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THE DEAD SPACE IN MODERATE AND LARGE RESPIRATORY VENTILATION *

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In a publication by one[1] of us, it was shown that in emphysematous patients (the minute volume and rate of respiration remaining the same) the concentration of carbon dioxid in the alveolar air increased when the degree of emphysema increased. It was also shown that this increased concentration of carbon dioxid in alveolar air was not caused by impairment of the function of external respiration, but was due to impairment of the ventilatory function of the lung. It was at first suspected that the dead space was increased in emphysema but when we collected the entire minute volume of air in a large rubber bag and took samples of alveolar air during the collection of the minute volume of air, we found that the dead space in patients who had severe emphysema was no larger than in normal persons. A large rubber bag 20 inches square was used for the purpose. The bag contains 60 liters of air before the tension of its walls will offer an appreciable resistance to expiration. Near the mouthpiece a lateral opening in the tube is provided for taking specimens of alveolar air.

The tube leading to the bag has a capacity of 120 c.c., so by clamping the tube near the bag and having the patient stop the end of the mouthpiece at the end of an expiration we could procure samples of alveolar air while the minute volume of air was collecting. The collected air was then passed through a meter and specimens of the expired air were taken for analysis while the air was measured with the meter.

The patient, S. G., had severe emphysema without asthma. Cyanosis was very pronounced.

Resp. rate = 20.5 per min.
Min. vol. = 8,200 c.c.
Expired air contained 4.38 per cent. of CO_2.

* Submitted for publication Oct. 1, 1914.

1. Hoover, C. F.: The Minute Volume and Alveolar Air in Pulmonary Emphysema, THE ARCHIVES INT. MED., 1913, xi, 52.

Three specimens of alveolar air contained 7.49 per cent., 7.86 per cent. and 7.00 per cent. of CO_2.
The mean CO_2 content of alveolar air = 7.45 per cent.
Each respiration = 400 c.c.
The alv. air = 235 c.c.
The dead space = 165 c.c.

Another test of S. G.'s respiration made in the same manner as above gave the following results:

Time of collection = 2 minutes.
No. of resp = 45.
Total expiration = 22,500 c.c.
Min. vol. = 11,250 c.c.
Each respiration = 500 c.c.
CO_2 in two specimens of alveolar air = 7.17 per cent. and 7.44 per cent.
CO_2 in expired air. Two specimens = 4.74 per cent. and 4.61 per cent.
Mean CO_2 for alveolar air = 7.30 per cent.
Mean CO_2 for expired air = 4.67 per cent.
Alv. air in each resp. = 319 c.c.
Dead space = 181 c.c.
Alveolar air = 63 per cent. of the min. vol.

On another trial when S. G. breathed 434 c.c. each respiration, the alveolar air contained 7.80 per cent. carbon dioxid and the total expired air contained 5.02 per cent. carbon dioxid. The dead space amounted to 155 c.c.

On all these occasions S. G. was cyanotic but suffered no sense of air hunger. When S. G. exercised we found the carbon dioxid content of his alveolar air was elevated so promptly and his ventilatory capacity was so limited that the dead space was not increased. When the estimates were made in the same manner employed by Haldane and Douglas, S. G. could increase his ventilation only by increasing the rate of respiration. He had no reserve expansion of the lungs which would increase the volume of each respiration; consequently his dead space remained unchanged with exercise, but the alveolar air carbon dioxid increased as much as 1.5 per cent. with moderate exercise. This increase of carbon dioxid percentage in his alveolar air permitted only very moderate walking. Severe exercise was unendurable.

Dr. G., a perfectly healthy young man, 5 ft. 8 in. high and weighing 140 pounds, breathed tranquilly while seated.

Time = 2 min. 2 sec.
No. of resp. = 29.
Total exp. = 25,750 c.c.
Each resp. = 887 c.c.
CO_2 in alveolar air { No. 1 = 5.33 per cent. / No. 2 = 4.91 per cent. / No. 3 = 4.78 per cent. } Mean = 5.00 per cent.
Expired air in CO_2 { No. 1 = 4.00 per cent. / No. 2 = 4.10 per cent.
Using mean alveolar air CO_2 of 5.00 per cent. and 4.10 per cent. CO_2 for the total expired air.
The alveolar air = 81 per cent. of the minute volume.
The dead space = 160 c.c.
Alveolar air of each resp. = 72 per cent.

Dr. G.'s dead space was measured many times while breathing tranquilly when seated and it was always found to measure between 150 and 180 c.c. We then made a series of experiments on Dr. G. and Dr. H. after the method employed by Haldane and Douglas and found the dead space increased as the volume of each respiration increased with exercise. We found, however, that with exercise the variations of carbon dioxid concentrations in our alveolar air were sufficient to give large variations in the estimated dead space when we were dealing with such large amounts for each respiration. We then suspected exercise had nothing to do with the large dead space but that the dead space enlarged because a large respiratory volume was not effective in diluting the alveolar carbon dioxid in direct proportion to the increasing volume of each respiration. Dr. G. breathed as deeply as possible five times and then began collecting the expired air.

Breathed 15 times in 1 minute and 40 seconds.
Total expired air = 48,880 c.c.
Each respiration = 3,258 c.c.
CO_2 in alveolar air { No. 1 = 3.59 per cent. / No. 2 = 3.69 per cent. / No. 3 = 3.47 per cent. } Mean = 3.58 per cent.
CO_2 in expired air { No. 1 = 2.87 per cent. / No. 2 = 2.93 per cent. } Mean = 2.90 per cent.
Alveolar air in each respiration = 2,666 c.c.
Dead space = 592 c.c.

Then in order that forced breathing should be avoided, Dr. G. breathed very slowly but very deeply. It was found that a deep respiration taken every 20 seconds was just sufficient to maintain respiratory comfort.

Dr. G. Breathed 5 minutes.
One respiration every 20 seconds.
Total respirations = 15.
Total expired air = 50,370 c.c.
Each respiration = 3,351 c.c.
CO_2 in alveolar air { No. 1 = 4.19 per cent. / No. 2 = 4.05 per cent. } Mean = 4.12 per cent.
Expired air CO_2 { No. 1 = 3.18 per cent. / No. 2 = 3.04 per cent. / No. 3 = 3.09 per cent. } Mean = 3.10 per cent.
Alveolar air in each respiration = 2,521 c.c.
Dead space = 830 c.c.

The same experiment repeated gave the following:

Time of collection = 5 minutes.
No. of respirations = 15.
Total expired air = 52,000 c.c.
Each respiration = 3,406 c.c.
CO_2 in alveolar air { No. 1 = 4.09 per cent. / No. 2 = 4.32 per cent. } Mean per cent. = 4.20.
CO_2 in expired air { No. 1 = 3.24 per cent. / No. 2 = 3.29 per cent. } Mean = 3.26 per cent.
Alveolar air in each respiration = 2,643 c.c.
Dead space = 763 c.c.

We then wished to see how the dead space would measure when Dr. G. breathed tranquilly but employed an excursion of the thorax which was just short of conscious effort. At this period of our research we were still of the opinion that a forced inspiratory effort dilated the bronchial tree after the infundibula were dilated. We suspected that the point of transition from tranquil breathing to conscious effort in lifting the thoracic cage would mark the point where the dead space increased.

Feb. 22, 1914, Dr. G. breathed tranquilly while seated. The breathing was just sufficiently deep so that he was not conscious of any effort at inspiration or expiration. Dr. G. said it was the upper limit of tranquil excursion, and larger inspiration would have required a conscious effort. After he had breathed in this manner for one minute, the collection of air was commenced.

Time of collection = 3 minutes.
Total expired air = 59,800 c.c.
No. of respirations = 46.
Each respiration = 1,300 c.c.
Min. vol. = 19,933 c.c.

Specimens of alveolar air were taken at the end of the first, second and third minutes.

CO_2 in alveolar air { No. 1 = 3.40 per cent. / No. 2 = 3.20 per cent. / No. 3 = 3.23 per cent. } Mean = 3.27 per cent.

CO_2 in expired air { No. 1 = 2.89 per cent. / No. 2 = 2.97 per cent. / No. 3 = 2.88 per cent. } Mean = 2.91 per cent.

Alveolar air in each respiration = 1,155 c.c.
Dead space = 145 c.c.

Feb. 26, 1914, Dr. G. breathed for one minute fifteen times with slightly conscious effort of expansion. Then the collection of air commenced.

Dr. G. Breathed every 5 seconds for 2 minutes, 5 seconds.
The total expiration = 52,500 c.c.
Each respiration = 1,810 c.c.

Specimens of alveolar air were taken 30 seconds apart.

CO_2 of alveolar air { No. 1 = 3.75 per cent. / No. 2 = 3.37 per cent. / No. 3 = 3.07 per cent. / No. 4 = 3.29 per cent. } Mean = 3.37 per cent.

CO_2 in expired air { No. 1 = 3.04 per cent. / No. 2 = 2.94 per cent. } Mean = 2.97 per cent.

Alveolar air in each respiration = 1,594 c.c.
Dead space = 206 c.c.

March 6, 1914, Dr. G. breathed deeply for one minute and then the collection of air was begun. Dr. G. breathed every ten seconds for three minutes. The expirations and inspirations were made as rapidly as possible. During the interim in which the breath was held, Dr. G.

held the glottis closed, so that from the end of a forced expiration until the beginning of the succeeding inspiration there could be no escape of carbon dioxid from the lungs.

Time of collection = 3 minutes.
No. of respirations = 18.
Total expired air = 5,760 c.c.
Each respiration = 3,202 c.c.

CO_2 in alveolar air { No. 1 = 3.67 per cent. / No. 2 = 3.30 per cent. / No. 3 = 3.39 per cent. } Mean = 3.45 per cent.

CO_2 in expired air { No. 1 = 2.61 per cent. / No. 2 = 2.65 per cent. } Mean = 2.63 per cent.

Alveolar air in each respiration = 2,440 c.c.
Dead space = 762 c.c.

On account of the varying percentages of carbon dioxid in the alveolar air we concluded to estimate the dead space by collecting the expired air of a single respiration and then compare the carbon dioxid content of the expired air with the carbon dioxid content of the end expiratory air of the same expiration.

For this purpose we used an 8-inch rubber bag. The bag contains 4 liters of air without offering any appreciable resistance to expiration.

A rubber tube leading to the bag contained 1 c.c. of water for every centimeter in length. The capacity of the tube measured with water was 120 c.c. A lateral opening in the mouthpiece enabled us to take samples of the end expiration air by clamping the tube at the bag and stopping the end of the mouthpiece at the end of a quick forced expiration. The results obtained by this method were quite the same as those procured by using the large bag. The larger the volume of respiration the larger the dead space found. This, however, did not settle the question, although it was growing more apparent that the great increase of the dead space in deep inspirations was an absurdity.

We then sought evidence of the comparative values of moderate and increasingly large respirations for dilution of the residual air in the lungs.

First, we wished to determine whether the amount of residual air was constant at the end of a forced expiration. When Dr. G. was seated he first made a forced expiratory effort and immediately followed the forced expiration with a forced inspiration. In each trial the expiratory effort was a maximum effort. The expired air from the maximum inspiration was captured in the small rubber bag and then measured by passing it through a meter. We found in many trials that the amount of air was very constant and varied only 200 c.c. The amounts measured always ranged between 3,600 c.c. and 3,800 c.c. This being the case, we are then justified in saying that in a normal person the amount of residual air in the pulmonary system at the

end of a forced expiration will be constant. Starting, then, with a constant residual air and employing a constant time for the initial expiration, a constant time for the inspiration and a constant time for the second expiratory effort, we have a method for measuring the dilution of the carbon dioxid in the residual air with varying inspiratory volumes. We first determined how much carbon dioxid per minute was being excreted.

April 17, 1914, two estimates were made. The first was made one hour after a full meal of proteins and carbohydrates, and the second estimate was made four hours after the meal. The specimens were procured by breathing into the large bag for five minutes while tranquilly seated. The two trials gave 417 c.c. of carbon dioxid moist per minute one hour after the meals. And the second trial four hours after the meal, gave a carbon dioxid excretion of 430 c.c. per minute. The lesser amount was employed, as that result was obtained shortly before the following experiments were made. We then started with the estimate of a carbon dioxid excretion of 6.95 c.c. per second. Dr. G. then made a forced expiration after breathing tranquilly for a few minutes while seated. This expiration was made through a tube 2 meters long fitted with a lateral opening in the mouthpiece so that a specimen of the end expiration air could be taken.

Then an inspiration was taken lasting two seconds. This was followed by a forced expiration lasting two seconds. The second expiration was made into the small bag fitted with a tube and mouthpiece which had a capacity of 120 c.c. At the end of the expiratory effort the tube was clamped at its junction with the bag and Dr. G. stopped the opening of the mouthpiece at the same instant. In this manner the end expiratory air of the second expiration was sampled and the total expired air was then thoroughly mixed by churning the bag and samples of the total expired air were taken from the lateral opening in the mouthpiece while the air was being passed through a meter. Knowing the exact dilution of the expired air with atmospheric air in the tube leading to the bag, we can accurately determine the carbon dioxid of the total expired air by estimating the carbon dioxid in the air contained in the bag.

We have then the following facts:

The excretion of carbon dioxid per second.

The carbon dioxid content of the residual air in the alveolar and dead space before ventilation.

The amount of ventilation.

The carbon dioxid in the expired air.

The carbon dioxid in the alveolar and dead space at the end of ventilation.

The time during that part of the procedure during which the excreted carbon dioxid was being contributed to the total expired air which was captured and its carbon dioxid determined.

The following are the results:

CO_2 excreted per second = 6.95 c.c.
Expired 1 second; alveolar air CO_2 = 6.06 per cent.
Inspired 2 seconds.
Expired 2 seconds; alveolar air CO_2 = 5.80 per cent.
CO_2 in expired air = 4.88 per cent.
Total expired air = 875 c.c.
Total alveolar air = 735 c.c.
Dead space = 140 c.c.

During the four seconds of inspiration and expiration 27.8 c.c. of carbon dioxid were contributed to the expired air.

We may assume the residual air of the lung to be 2,000 c.c., which is certainly a liberal estimate.

The dead space estimated in the experiment was 140 c.c. and as the volume of the respiration was very moderate we may assume the dead space unchanged during the experiment.

At the end of the first expiratory effort the total carbon dioxid in the pulmonary system can be expressed by the formula (2,000 + 140) 6.06 per cent.

If we assume there were no carbon dioxid excreted during the following four seconds, then the carbon dioxid content of the alveolar and dead spaces at the end of the inspiratory effort may be expressed by 2,000 + (875 — 140) multiplied by x. We then have the formula:

$$\frac{2140 \times 6.06\%}{2735} = x \text{ or } 4.74\%$$

This is the percentage of carbon dioxid we should have obtained in the alveolar air at the end of the second expiration had no carbon dioxid been excreted in the interim of four seconds. But 27.8 c.c. of carbon dioxid were contributed to the air in the infundibula and alveolar spaces. In this instance that amount of air equaled 2,735 c.c.

$$27.8 = 1.01\% \text{ of } 2{,}735$$

Therefore to the theoretically ventilatory result of 4.74 per cent. carbon dioxid we add the contributed carbon dioxid amounting to 1.01 per cent., and this amounts to 5.75 per cent., whereas the actual estimate made was 5.80 per cent.

Therefore in moderate respiratory amounts the dilution of the residual air carbon dioxid can be completely accounted for.

How is the dilution of residual air when large respiratory amounts are employed?

About two hours after the foregoing experiment was performed Dr. G. performed the following experiment:

Expired 1 second the alveolar air CO_2 = 6.23 per cent.
Inspired 3 seconds.
Expired 2 seconds alveolar air CO_2 = 5.09 per cent.
Amount expired air = 2,810 c.c.
CO_2 in expired air = 4.12 per cent.
Total alveolar air = 2,274 c.c.
Dead space = 536 c.c.

The total carbon dioxid content in the tracheobronchial and alveolar spaces at the end of the first expiration = (2,000 + 140) 6.23 per cent.

If we accept the enlargement of the dead space to 536 c.c., as our results showed, then at the end of the deep inspiration the dilution of the carbon dioxid in the alveolar air is expressed by the formula:

$$2{,}000 + (2{,}810 - 536) \text{ times } x$$

From this we have the formula:

$$\frac{2{,}140 \times 6.23\%}{4{,}274} = x \text{ or } 3.11\% \text{ of } CO_2$$

This is the concentration of carbon dioxid in the alveolar air had there been no excretion of carbon dioxid during the respiration, which occupied five seconds.

Using the maximum amount of carbon dioxid found in the minute volume of air, namely: 430 c.c. during the five seconds 35.8 c.c. of carbon dioxid were excreted, which amounts to 0.83 per cent. of 4,274 c.c., which was the minimum amount of air in the alveolae and infundibula if we assume the deep inspiration enlarged the dead space. Therefore, adding 0.83 per cent. to 3.11 per cent., we have only 3.94 per cent., the amount of carbon dioxid we should have found in the alveolar air at the end of the deep respiration had the deep respiration been effective in proportion to the amount of the total inspiration. The concentration really found at the end of the deep respiration was 5.09 per cent. carbon dioxid. So the disparity between the actual and the theoretical dilution was 1.15 per cent. This can be accounted for only by the want of equal diffusion of gases in the infundibular and alveolar spaces when the lung is greatly distended.

Another method of treating the results algebraically shows how the effectiveness of dilution of the residual air diminishes with the increasing volume of respiration.

In our results the residual air is the only unknown factor. When treated in this manner we evolve the following formula:

First experiment, April 17.
Let x = the residual air in the alveolae.
Dead space = 140 c.c.
CO_2 concentration of alveolar air and dead space air at the end of the first expiration in Experiment 1 = 6.06 per cent.

CO_2 at the end of the first expiration = (x + 140) 6.06 per cent. in the alveolae and dead space.

At the end of the inspiration the total CO_2 in the alveolae = x + (875—140) times 5.80 per cent.

But 27.8 c.c. of CO_2 are excreted during the four seconds, therefore

$$(x + 140)\ 6.06\ \% + 27.8 = (x + 735)\ 5.80\ \%$$
$$x\ 6.06\ \% + 36.28 = x\ 5.80\ \% + 42.63$$
$$(6.06\ \% - 5.80\ \%)\ x = 42.63 - 36.28.$$
$$x = \frac{42.63 - 36.8}{6.06\% - 5.80\%}$$
$$x = \frac{5.83}{.0026}$$

x or residual air = 2,203 c.c., which is a very close estimate of the residual air.

If we employ the results of Experiment 2 of April 17 to estimate the residual air we will have the following formula:

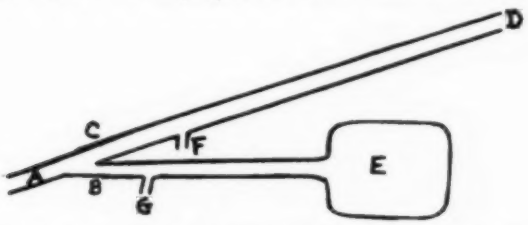
x = residual air.

CO_2 secreted in 5 seconds = 35.8 c.c.

$$(x + 140)\ 6.23\ \% + 35.8 = (x + 2{,}274)\ 5.09\ \%$$
$$x\ 6.23\ \% + (140 \times 6.23\ \%) + 35.8 = x\ 5.09\ \% + (2{,}274 \times 5.09\ \%)$$
$$x\ 6.23\ \% + 44.522 = x\ 5.09\ \% + 115.7466$$
$$x\ 6.23\ \% - x\ 5.09\ \% = 115.746 - 44.522$$
$$(6.23\ \% - 5.09\ \%) = 71.224$$
$$x = \frac{71.224}{0.0114}$$

x or the residual air = 6,274 c.c.

This is of course absurd but should be true if the large ventilation diluted the residual air in proportion to the volume of ventilation.



In the figure A is a rubber tube which has the same lumen as its two branches B and C; E is a rubber bag with a capacity of 4,500 c.c.; F and G are lateral openings through which samples of the alveolar air may be procured.

April 25, an experiment was made to see what would be the actual effect of ventilation in a normal person when the dead space was artificially prolonged. The apparatus used consisted of the device illustrated in the accompanying figure.

Dr. G. breathed quietly while seated. After breathing for several minutes to obtain perfectly tranquil and comfortable ventilation, he then made a quick, forced expiration lasting one second. During this time a clamp was applied at B so all expired air passed through the tubes A, C, D. The tube C was 50 cm. longer than the tube B (which was 275 c.c. long), because at the end of the forced expiration (lasting one second) a sampling tube with a capacity of 50 c.c. was filled with

alveolar air. The rubber tubing had a capacity of 1 c.c. for each centimeter in length. Then, at the beginning of the inspiration, which was drawn through the tube A, C, D, the prolongation of the dead space was the same as the prolongation of the dead space for the following expiration, which was made through the tube B (with C clamped). At the end of the second expiration a sample of the alveolar air was taken at G. The expired air was captured in the bag E.

The following were the results of the experiment:

Expiration 1 second alveolar air CO_2 = 6.26 per cent.
Inspiration 3 seconds.
Expiration 2 seconds alveolar air CO_2 = 6.53 per cent.
The expired air contained = 4.28 per cent. CO_2
Total alveolar air = 1,065 c.c.
Dead space = 560 c.c.

We see when the dead space is actually prolonged 275 cm., a respiration of 1,625 c.c. was not sufficient to dilute the alveolar air, but resulted in a further concentration from 6.26 per cent. carbon dioxid to 6.53 per cent. carbon dioxid.

This result was confirmed by repetitions of the experiment and merely affords added proof against the dead space being enlarged with an increased volume of respiration.

The results of our studies of lung ventilation are quite consistent with Oppel's studies of the functional anatomy of the lung. Keith,[2] quoting Oppel, says:

> If out of the rubber balloon a model of the terminal bronchiole infundibulum and alveoli be made and inflated, it is the central or infundibular space which expands most, the alveoli implanted on its walls being widened but at the same time rendered more shallow. The point which one seeks to emphasize is that it is not the alveoli but the infundibula that should be regarded as the essential expansile parts of the lung.

From these results it is quite apparent why we must employ such a large minute volume of air to lower the carbon dioxid concentration of the alveolar air in forced breathing.

This conception also explains the respiratory limitations in physical exercise.

It is also a protective measure for maintaining a constant concentration of carbon dioxid at the respiratory membrane.

Thus far we have always spoken of ventilation of the lung as a process in which the alveolar air is diluted by the tidal air. This is true in one sense, but we have lost sight of another view of lung ventilation which the foregoing experiments strongly suggest. Lung ventilation amounts to concentration of the carbon dioxid of alveolar air into the bubble of tidal air which enters the atrium with each

2. Hill: Further Advances in Physiology, p. 186.

inspiration. Ventilation also means a concentration of the oxygen of the tidal air into the alveolar air.

When we take this view of the lung ventilation it is apparent why ventilation of a lobule with a large bubble of air does not secure a diminution of the carbon dioxid concentration in the alveolar air proportionate to the volume of ventilation. Our results prove that when from 700 to 1,300 c.c. are inspired, there is a concentration of carbon dioxid in the inspired air which equals that of the air at the respiratory membrane or at least so nearly so that our methods enable us to account for the effectiveness of a known volume of ventilation in diluting the alveolar air carbon dioxid when these moderate amounts of ventilation are employed. But when large amounts of air are employed (for instance, 3,600 c.c.), then the bubble of air in the atrium is too large to procure a concentration of carbon dioxid in the bubble of ventilating air which will equal or nearly equal the concentration of carbon dioxid that exists at the respiratory membrane. The reason for this seems to lie in the fact that there is not sufficient time for this to be accomplished. The act of respiration is continuous. The transition from the inspiratory to the expiratory phase occurs in so brief a time that when the ventilating bubble is of considerable size there is not sufficient time to procure a uniform concentration of carbon dioxid in all the air contained in a lobule of the lung. With this idea in mind we made further experiments to see what effect the arrest of respiration at the end of an inspiration would have on the size of the dead space and on the concentration of carbon dioxid in the alveolar air.

Dr. G. breathed tranquilly (while seated) into the large rubber bag.

Time of collection = 5 minutes.
Total expiration = 66.650 c.c.
CO_2 in expired air = 4.10 per cent.
CO_2 per minute = 534.53 c.c.

Dr. G. breathed quietly and then at the end of a normal inspiration he made a forced expiration lasting one second. A specimen of alveolar air was taken at the end of this expiratory effort. Then G. took a moderate inspiration and held his breath with the glottis closed for ten seconds; then he expired two seconds and a specimen of alveolar air was taken at the end of this expiratory effort. The total expiration was also captured. There was then an interval of twelve seconds during which the excreted carbon dioxid was being concentrated in the alveolar air.

The following results were procured:

First alveolar air CO_2 = 5.75 per cent.
Total expired air = 820 c.c.
CO_2 in the expired air = 5.72 per cent.
Second alveolar air CO_2 = 6.85 per cent.
Total alveolar air = 684 c.c.
Dead space = 136 c.c.

Applying the same formula we employed in former experiments.

(2,000 + 150) 5.75 % = (2,000 + 820 — 134) x
2,150 × 5.75 per cent. = 2,684 times x
x = 4.60 per cent.

The amount of carbon dioxid in the alveolar air would have been 4.6 per cent. had there been no carbon dioxid excreted during the interval of twelve seconds. The amount of carbon dioxid given off in twelve seconds = 106.90 c.c. and 106.90 = 3.98 per cent. of 2,684. Therefore the concentration of carbon dioxid in the alveolar air would have been 4.60 per cent. + 3.98 per cent. = 8.58 per cent. if the carbon dioxid had not been stored in the blood. The amount of carbon dioxid found in the alveolar air at the end of the second expiration was 6.85 per cent. This experiment shows how promptly the carbon dioxid accumulates in the fluids of the body. It is true the dead space was small, but under the circumstance of arrested breathing we cannot assume that there was the same differentiation between alveolar air and dead space as in uninterrupted breathing. For this reason we cannot accept the estimate of the dead space as correct. We then tried holding the breath for thirty seconds with the following results:

Alveolar air end of expiration = 5.76 per cent. CO_2.
Inspired and closed glottis for 30 seconds.
Expiration 2 seconds alveolar air = 7.25 per cent. CO_2.
Total expired air = 620 c.c.
CO_2 in expired air = 6.07 per cent.
Total alveolar air = 510 c.c.
Dead space = 110 c.c.

Employing the same formula we find the carbon dioxid concentration in the alveolar air we would have found had there been no carbon dioxid excreted during the interval of 32 seconds, was 4.93 per cent. Carbon dioxid excreted in 32 seconds = 284.80 c.c. and this amounts to 11.34 per cent. of 2,510 c.c. So we should have found 16.2 per cent. of carbon dioxid were the carbon dioxid not promptly stored in the fluids of the body as the percentage of carbon dioxid rises in the alveolar air.

On another occasion G. breathed tranquilly and we found he was exhaling 410 c.c. of carbon dioxid per minute.

A forced expiration lasting 1 second was made and the alveolar air contained 6.33 per cent. carbon dioxid.

G. inspired and held the glottis closed for 30 seconds.

Then G. expired 2 seconds.

The alveolar air CO_2 = 7.33 per cent.
Amount of expired air = 1,195 c.c.
CO_2 in expired air = 6.46 per cent.
Total alveolar air = 1,053 c.c.
Dead space = 142 c.c.
(2,000 + 142) 6.33 per cent. = (2,000 + 1,195 — 142) x

Therefore x = 4.44 per cent. or the percentage of CO_2 we would have found were there no CO_2 excreted during the interim of 32 seconds.

But during this interim 218.56 c.c. of carbon dioxid were excreted and this amounts to 7.15 per cent. of 3,053. Therefore, were the carbon dioxid not promptly stored in the body fluids we would have obtained 11.59 per cent. carbon dioxid instead of 7.33 per cent. in the alveolar air.

SUMMARY

1. The optimum of respiratory volume is about 1,200 c.c. When the respiratory volume is increased beyond this amount the effectiveness of lung ventilation does not increase proportionately with the increased ventilation.

2. It is the want of diffusion of carbon dioxid in large respiratory excursions which has given rise to the error of supposing the dead space to be increased in exercise.

3. We have shown on the basis of measured ventilation and the results in diluting the residual air, that forced breathing is not effectual in ventilating the lungs in proportion to the increasing amounts of ventilating air. And conversely, when the amount of residual air is estimated on the basis of ventilatory results, the amount of residual air can be estimated with some accuracy when moderate amounts of air are inhaled, but when forced breathing is employed the residual air cannot be estimated from the measured results of ventilation.

4. When the dead space is artificially increased, the results of ventilation are not at all consistent with the ventilatory results which are ascribed by other investigators to an increase in the dead space in large respiratory excursions.

5. The inefficiency of large respiratory excursions contributes more to the limitations of endurance in exercise than do limitations in the circulatory system.

6. The foregoing results also prove the manner in which operative interference contributes to the relief of emphysema. Relief is not procured by diminishing the amount of residual air, whose carbon dioxid is diluted by respiration. Improvement is procured from the operation by restoring the ventilatory effectiveness of moderate respiratory volumes, the improvement from Freund's operation is due to diminution of the size of the lobules of the lung and not to changes in rigidity of the chest wall.

THE OCCURRENCE OF NUCLEAR PARTICLES IN THE ERYTHROCYTES FOLLOWING SPLENECTOMY*

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Recently O. Roth[1] reported unusual blood findings in a patient whose spleen had been removed twelve years before, because of supposed splenic anemia.† Numerically, the red corpuscles of Roth's patient varied between 5,600,000 and 6,468,000, the leukocytes between 8,900 and 16,000, while the hemoglobin fluctuated between 100 and 114 per cent. Thus, there was no anemia.

The microscopic examination of the fresh and stained specimens of blood revealed, as Roth points out, striking abnormalities. In the fresh specimen, it was evident that a large part of the erythrocytes presented one to two round, quite refractive inclusions. Generally, they were the size of a very small coccus, but at times the diameter amounted to one-sixth of that of the red cell. They moved slightly in the cell. When the blood was stained with Giemsa's mixture, it was seen that these bodies were structureless, sharply outlined and were stained red; they possessed all the characteristics of the Howell bodies. Usually one only, rarely two, bodies were observed in an erythrocyte. On one occasion Roth estimated that 20,000 red cells per 1 c.mm. contained nuclear particles or Howell's bodies.

Furthermore, in the stained blood, about one-half of the red corpuscles presented fine dots, which were stained blue with Giemsa's stain. The dots were usually single, though as many as three were found in a cell. Many of the cells containing a nuclear particle also contained dots.

Roth attributes the blood changes to the loss of the spleen.

In 1908, Schur[2] reported a similar case, in which he suspected the relationship between lessened splenic function and the occurrence of nuclear particles in the erythrocytes. His patient had had Basedow's

* Submitted for publication Aug. 22, 1914.
* Presented before the American Society for the Advancement of Clinical Investigation, Washington, D. C., May, 1913.

1. Roth, O.: Ueber merkwürdige Erythrocyten-Einschlüsse bei einem Fall von Milzextirpation, Ztschr. f. klin. Med., 1912, lxxvi, 23.

† Roth is convinced that the patient was suffering from congenital hemolytic jaundice at the time of operation.

2. Schur, Heinrich: Ueber eigenartige basophile Einschlüsse in den roten Blutkörperchen bei einem Fall von abgelaufenem Morbus Basedowii mit nachfolgender schwerer makrocytischer Anämie. Wien. med. Wchnschr., 1908, lviii, 442-511.

disease for ten years. She first consulted him in 1905; at this time the symptoms were mild. Blood examination showed 4,500,000 erythrocytes, 10,000 leukocytes, and 80 per cent. hemoglobin. In the fresh blood there was seen, in many of the red cells, a single refractive body, usually eccentric, and often moving in the cell. In the stained films these bodies were markedly basophilic. They were stained with all nuclear dyes, especially well with Giemsa's and with Romanowsky's stains. The bodies were least evident in preparations stained with Ehrlich's triacid stain, only a part of them staining green, while others were evident by a deeper staining at their periphery. The bodies were nearly always perfectly round; at times they appeared to be ring-like. There was never a definite structure to the bodies. At times their diameter amounted to one-fourth that of the red corpuscle. Generally, they were smaller, down to a minute point. The bodies were usually single; never more than two were found in a cell. Early in 1907 a severe anemia of the pernicious type developed, and in November the patient died. At autopsy the spleen was found to be 10 cm. long, 3 cm. wide at the upper end, only 1 cm. wide in its lower one-half. There was no thrombosis of the splenic vessels. Microscopically, the splenic pulp was found to be scanty; connective tissue predominated.

The peculiar inclusions in the erythrocytes, which were still present shortly before death, Schur identifies with the similar bodies observed by Howell[3] in the blood of the cat. In view of the severe anemia which developed, he looked on them as an expression of a degenerative change in the erythrocytes.

Schur believed the occurrence of the nuclear particles was in some way connected with the splenic atrophy, although, he says, extirpation of the spleen is not followed by marked changes in the erythrocytes, either in man or animals. He believed that the change in the spleen was not the primary factor, but that the atrophy of the spleen and the appearance of nuclear particles in the red cells were both probably due to some unknown toxin.

For some years I have been interested in the study of nuclear particles in the erythrocytes, especially their occurrence in human blood. In a previous report,[4] the blood of one patient whose spleen had been removed was noted. It was described (p. 95) in the following words:

> In some bloods, however, single nuclear particles in the erythrocytes may be the main evidence of regeneration; this fact was well illustrated in the case of the patient following splenectomy, in whose blood, at one time (i. e.,

3. Howell, W. H.: The Life-History of the Formed Elements of the Blood, Especially the Red Corpuscles, Jour. Morphol., 1890, iv, 57.

4. Morris, R. S.: Nuclear Particles in the Erythrocytes, THE ARCHIVES INT. MED., 1909, iii, 93.

thirteen days after splenectomy), thirty nuclear particles were found in about an hour and a half (examination with mechanical stage), while only one normoblast, no cells with basophilic granules, and a few with polychromatophilia were seen.

Roth's report led to a reexamination of the stained smears of the patient just referred to. The result of this, together with the findings in two other specimens of blood from splenectomized individuals, form the basis of the present report.

TECHNIC

All the bloods were stained with a Romanowsky stain (Giemsa's, Wilson's), as nuclear particles are best demonstrated in this way. The slides were examined with the $\frac{1}{12}$" oil immersion objective, ocular No. III (Leitz), a mechanical stage being employed. To narrow the field, a circular piece of paper, in the center of which a small square was cut, was inserted in the eye-piece. As nuclear particles are minute and are readily overlooked, it is essential, particularly when they are scarce, to move the stage very slowly, in order that all parts of the field may be carefully scrutinized.

CASE 1.—Patient of Dr. W. S. Thayer of Baltimore. The specimens, stained originally in November, 1908, with a Romanowsky stain, were faded. The cover slips were removed from the slide with xylol and in October, 1912, they were restained with Giemsa's stain (1 drop to 5 c.c. of water, allowed to act forty-eight hours). In a specimen obtained from the patient thirteen days after splenectomy, the red blood cells showed moderate anisocytosis and slight poikilocytosis. Many of the cells were pale. In counting 500 leukocytes, there were found 130 red corpuscles containing nuclear particles. Two cells were observed with two nuclear particles each, while one contained three; all the remaining cells presented only a single nuclear particle. The nuclear particles varied considerably in size and were almost always eccentrically situated in the cell. During the examination, three normoblasts and one erythrocyte containing basophilic granules were found. Chromatin dots (Weidenreich's *Chromatin-Staübchen*) were fairly numerous but less so than the nuclear particles. The leukocytes were poorly differentiated. Blood platelets were apparently greatly increased in number and giant platelets were not uncommon.

In a smear from the same patient made three days later (i. e., sixteen days after splenectomy), only eighteen erythrocytes with nuclear particles were found; in all instances the nuclear particle was single and usually eccentrically situated. No erythroblasts were seen and only two cells with basophilic granules were encountered. Chromatin dots were much more numerous. A considerable percentage of the erythrocytes contained them. Their number varied from one to three in a cell; they were usually placed near the periphery of the corpuscle. They were stained reddish purple, like nuclei and nuclear particles. A differential count in this specimen (500 cells) gave: lymphocytes, 10.4 per cent.; large mononuclears and transitionals, 16 per cent.; polynuclear neutrophils, 64.4 per cent.; eosinophils, 7.6 per cent.; mast cells, 1.6 per cent.[5]

CASE 2.—Patient of Dr. Vilray P. Blair of St. Louis. A splenectomy was performed Nov. 15, 1912. Death occurred five days later. Smears of the

5. As blood counts were not made on the days that smears were prepared in this or the following cases, it is impossible to estimate, with any degree of accuracy, the number of cells per c.mm. containing nuclear particles.

patient's blood were made a few hours before death. The specimens were stained with Wilson's stain. Examination revealed ten erythrocytes with nuclear particles in counting 250 leukocytes. Most of the nuclear particles were of good size. During the examination one normoblast containing basophilic granules was seen. There was slight polychromasia, and a few cells contained chromatin dots.

CASE 3.—A specimen of blood from a patient a few days after splenectomy, was received from Dr. S. R. Miller of Baltimore. In counting 200 leukocytes, thirty-eight erythrocytes containing nuclear particles were observed, all single and of fair size. No normoblasts or cells with basophilic granules were found, nor was there polychromasia, anisocytosis or poikilocytosis.

My three cases with Roth's make four in whose blood nuclear particles have been found following splenectomy. In Schur's case, the great abundance of the nuclear particles with the marked splenic atrophy found at autopsy is suggestive of a causal relationship, in the light of the similar blood findings in Roth's case. In Roth's patient the nuclear particles were abundant years after splenectomy; in all of my cases the nuclear particles, though much less numerous, had appeared within two weeks after operation — in one case, indeed, within five days. It is unfortunate that we do not know whether nuclear particles were present or absent in the blood before operation.

Musser[6] reviewed the blood findings in man following splenectomy as reported in the literature. In no case does he mention the occurrence of nuclear particles. In the blood of three splenectomized dogs, he also makes no mention of nuclear particles.

The relationship between the occurrence of nuclear particles in the erythrocytes and complete (or partial) loss of splenic function, if such there be, remains to be demonstrated. The results in my three cases and in Roth's are suggestive. It is quite possible that it may be found that this is a characteristic blood change following removal of the spleen: for, unless one's attention were directed to them, it would be difficult to detect scattered nuclear particles in the course of the usual examination. It is, of course, not suggested that the finding of nuclear particles in the erythrocytes means absence of splenic function, for nuclear particles are not uncommon in various diseases of the blood.

6. Musser, Jr., J. H.: An Experimental Study of the Changes in the Blood Following Splenectomy, THE ARCHIVES INT. MED., 1912, ix, 592.

AGE INCIDENCE IN SARCOMA*

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In a previous statistical study[1] it was shown that the incidence of carcinoma is greatest at the age period 58 to 62, and that there is a definite decrease in carcinoma incidence after this period. In the present report it is proposed to treat by a similar method a large group of sarcoma ages obtained from the same source, hoping in this way to contribute to the knowledge of the biologic relationship between the two types of malignant disease.

In the past it has been the custom of both pathologists and surgeons to find in age incidence one of the chief points of difference between carcinomas and sarcomas. The usual statement of text-books has been that carcinoma is a disease of the old and sarcoma of the young. Da Costa[2] says that sarcomas may arise at any period from birth to extreme senility, but that they are commonest during youth and early middle age. On the other hand, Williams several years ago,[3] and again more recently,[4] has declared that this differentiation between carcinoma and sarcoma cannot be made. His conclusions are quoted in full:

> The sarcomata may arise at any period of life; a certain number of cases are congenital; more are met with in early infancy, especially during the first five years, than at any other period prior to the twentieth year; after which sarcomata increase in frequency until middle life, becoming rarer again in old age.
>
> It will be gathered from what I have stated that there are close analogies between the two diseases — sarcoma and carcinoma — such differences as are noticeable being due to diversity of origin and its consequences, rather than to any essential difference in the nature of the morbid process.

Williams found that he could derive the same conclusion either from the onset ages as taken from hospital records or from mortality returns.

As will be seen, our analysis of 265 consecutive cases of sarcoma bears out these conclusions in practically every particular. The method which has been used is the same as that employed in the preparation of the previous report[1] on age incidence in carcinoma. Therefore, it

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* Submitted for publication Aug. 14, 1914.

* From the Department of Pathology, University of Michigan.

1. Weller: Age Incidence in Carcinoma, THE ARCHIVES INT. MED., Chicago, 1913, xii, 539-545.
2. DaCosta: Modern Surgery, 1910, p. 365.
3. Williams: Twentieth Century Practice of Medicine, 1898, xvii, 487.
4. Williams: The Natural History of Cancer, 1908, p. 323.

will not be necessary to repeat it in detail here. The material from which these figures were derived consists of the consecutive cases of sarcoma of known age diagnosed in the pathological laboratory of the University of Michigan between the years 1895 and 1913. The advantages which this material possesses over either cancer censuses or mortality returns may again be briefly outlined. These data are derived

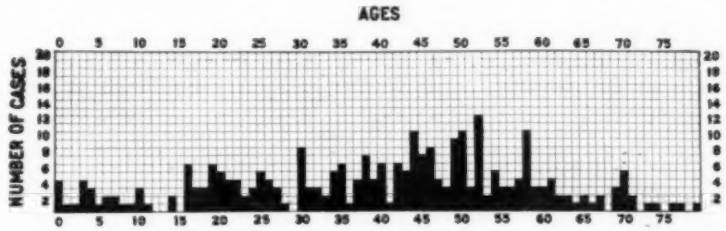

Chart 1.—Age distribution of sarcoma.

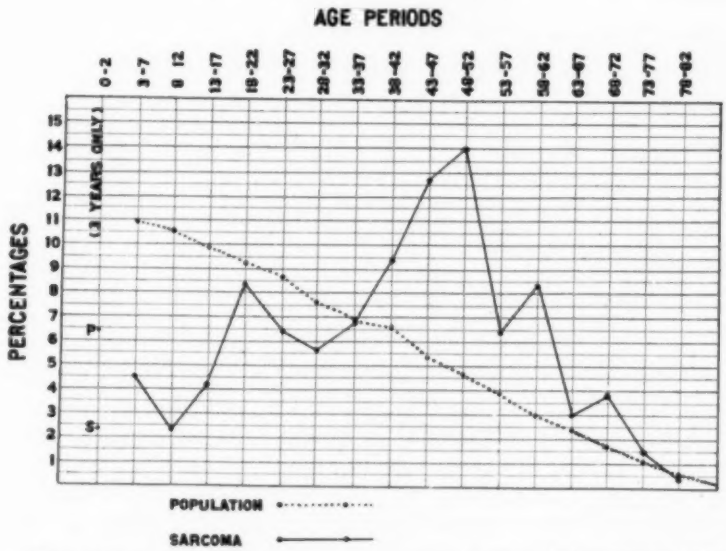

Chart 2.—Percentage of sarcoma cases for each age period contrasted with the percentage of total population living at each age period.

from a representative fraction of the population of Michigan and the neighboring states; the ages are given at an earlier stage of the disease than is possible in either of the other types of statistics; and each case has had complete microscopical verification. The figures for the age distribution of the total population are taken from the United States

Census Report[5] for 1900. Since less than 10 per cent. of these cases of sarcoma came from without the state of Michigan, it has been possible to use the age distribution of the population of Michigan alone without introducing any appreciable error.

The yearly distribution of the ages of the total 265 cases of sarcoma is shown by Chart 1. As was the case with a similar chart showing carcinoma ages, the years which are multiples of five, and also, in a lesser degree, the even years, are favored by patients in giving their ages. For instance, there are ten cases at age 50 and twelve at age 52, while only three patients gave 51 as their age and only two are found at 53. This error of approximate answers has been overcome almost entirely in all subsequent charts by the simple device of grouping all

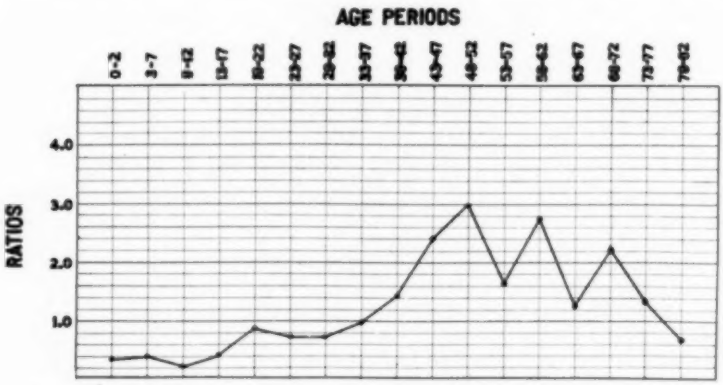

Chart 3.—Corrected curve of age incidence of sarcoma obtained by plotting a series of ratios between the percentages used in Chart 2.

figures in age periods of five years each with the multiple of five as the middle year in each group. From Chart 1 it should be noted that the number of cases of sarcoma is comparatively small until age 16 is reached, and that the greatest number of cases in any one year falls at age 52.

In Chart 2 the percentage of the total number of sarcoma cases for each age period is graphically contrasted with the percentage of the total population which is living at each age period. The sarcoma curve lies below the population curve until age 35 is reached. It then remains above the population curve until extreme old age is reached, at which period the proportion of the population found to be living and the number of sarcoma cases alike become too small to permit of drawing

5. Report, Twelfth Census, U. S., 1900, ii, Part 2, p. 52.

trustworthy conclusions. Stated in another way, these curves indicate that up to age 35 the incidence of sarcoma is less than it would be were sarcoma uniformly distributed throughout the entire population; that at age 35, the point of crossing of the two curves, the incidence of sarcoma is the same as it would be were sarcoma uniformly distributed throughout the total population, and that after age 35 sarcoma is much more prevalent than a uniform distribution would lead one to expect. Now the curve of *carcinoma* percentages was found to cross the curve of population percentages at age 37, and thus there is found to be an almost exact coincidence of the points of crossing in the two types of malignant disease. This close analogy in respect to age distribution will be more completely demonstrated in another chart.

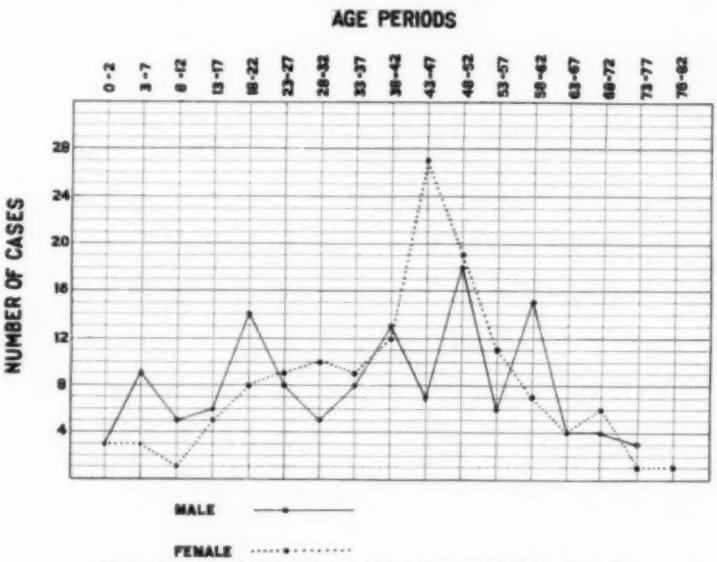

Chart 4.—Age distribution of sarcoma divided as to sex.

In Chart 3 the data embodied in Chart 2 are handled in a somewhat different way. The sarcoma curve of Chart 2 does not in itself give a true curve of age incidence for sarcoma, because of the decreasing population in each successive age period. In Chart 3 a corrected curve of age incidence is obtained by plotting a series of ratios between the percentages used in Chart 2. For instance, the

$$\text{Ratio for age period N} = \frac{\text{percentage of sarcoma for age period N}}{\text{percentage of population for age period N}}$$

From the true curve thus obtained it appears that the incidence of sarcoma is relatively low until the period of puberty is reached. At

that time there is a moderate increase, and this higher level is maintained until age 30. There is then a rapid rise until age period 48 to 52 is reached, at which point is found the maximum incidence of the disease. After this period the ratios again decrease until they become less than one in extreme old age. The fluctuations of this portion of the curve are due in part to the marked degree in which patients favored the multiples of ten in giving their ages, and in part to the smaller number of cases to be found at each age period, since with figures for the number of cases and for the population both relatively very small, slight variations cause correspondingly greater fluctuations in the curve.

If sarcoma were uniformly distributed in respect to age incidence, the ratio for each age period would be unity. This chart (No. 3) shows that the actual incidence is below this level throughout the first

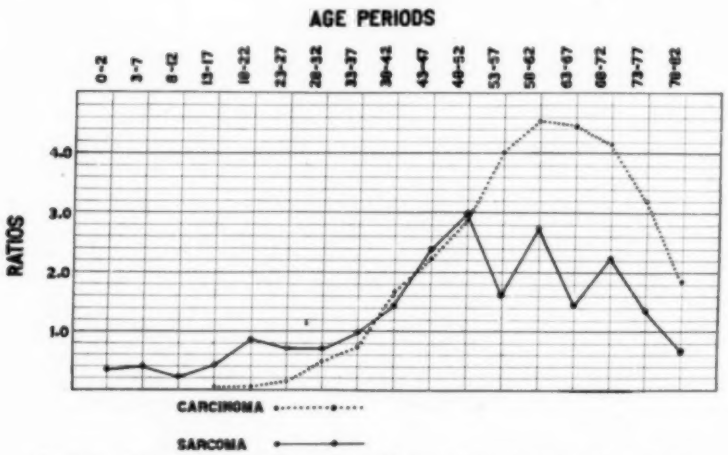

Chart 5.—Corrected curves of age incidence of carcinoma and sarcoma.

half of life and above unity throughout the second half of life. It is, therefore, impossible to speak of sarcoma as a disease of the young. It is frequent in the young, but most common in late middle life.

In Chart 4 the age distribution of our sarcoma cases, divided as to sex, is shown. Of the total 265 cases, 129 occurred in males and 136 in females. When these are arranged according to age periods, there is a rough parallelism between the two curves. This would doubtless be more perfect if a larger number of cases were under consideration. The apex of the curve of cases in females is at age period 43 to 47. This is of interest for the apex of the curve of female carcinoma cases also occurred at this age period. Unlike carcinoma, however, these

curves do not definitely indicate any earlier incidence of sarcoma in females than in males. This was to have been expected, for in the previous report it was shown that the earlier cycle of carcinoma incidence in females is largely determined by the carcinomas of the breast and uterus, organs which early undergo involution changes. These two sources provided more than two-thirds of all our cases of carcinoma in women. In the anatomical distribution of sarcoma no such powerful factors are introduced.

On Chart 5 are plotted the fully corrected curves of age incidence of carcinoma and of sarcoma. The carcinoma curve is reproduced from the report to which reference has previously been made. The great similarity of the two curves is evident, but the most striking feature is the almost perfect coincidence of the two curves from age period 28 to 32 up through age period 48 to 52. Although the sarcoma incidence in the young is higher than the carcinoma incidence, it still remains less than unity, and so age incidence, instead of affording a means of contrast or differentiation, serves but to emphasize the biologic similarity between the two great types of malignancy.

In conclusion, the analysis here given indicates that:

1. The incidence of sarcoma is greatest at the age period 48 to 52. Therefore, sarcoma can no longer be considered a disease of the young.

2. After this period the sarcoma incidence gradually decreases.

3. There is no marked difference in the age distribution of sarcoma in males and in females.

4. Although in youth sarcoma incidence is somewhat higher than carcinoma incidence, there is throughout life a marked parallelism between the age incidence curves for the two types of malignancy, and for more than twenty years there is a practical coincidence, thus strongly suggesting that the causal or predisposing agencies in the two cases must either be identical or, at least, have much in common.

STUDY XXIII. THE RELATION BETWEEN AMYLASE RETENTION AND EXCRETION AND NON-PROTEIN NITROGEN RETENTION IN EXPERIMENTAL URANIUM NEPHRITIS*

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Tests for renal function fall into three general groups depending on: (1) The ability of the kidney to excrete from the circulation abnormal substances, such as dyes or drugs which are introduced into it; (2) analysis of the products of metabolism retained in the blood as a result of faulty eliminative powers of the kidney; (3) abnormalities in the physical and chemical properties of the urine.

The phenolsulphonephthalein test is an excellent example of the first group. Rowntree and Geraghty[1] have found that the amount of this dye excreted in the urine after its intramuscular or intravenous injection varies almost in proportion to the degree of injury existing in the kidneys. This test is used so generally by clinical and experimental observers that its value in the study of the renal function can be accepted without further comment.

Of substances studied in the blood of nephritics the concentration of the non-protein nitrogen and urea is of most importance. Among others Ascoli,[2] Strauss,[3] Obermayer and Popper,[4] Widal[5] and Foster[6]

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* Submitted for publication Sept. 29, 1914.

* From the Laboratory of the Theory and Practice of Physic, Harvard Medical School, and the Medical Clinic of the Peter Bent Brigham Hospital.

* This is one of a series of studies on experimental cardiorenal disease: Study I, Smith: Boston Med. and Surg. Jour., 1908, clviii, 696; Study II, Christian: Boston Med. and Surg. Jour., 1908, clix, 8; Study III, Christian: Jour. Am. Med. Assn., 1909, liii, 1792; Studies IV-XV, Christian, Smith and Walker: THE ARCHIVES INT. MED., 1911, viii, 468-551; Study XVI, Christian and O'Hare: THE ARCHIVES INT. MED., 1913, xi, 517; Studies XVII and XVIII, O'Hare: THE ARCHIVES INT. MED., 1913, xii, 49, 61; Study XIX, Christian and O'Hare: Jour. Med. Research, 1913, xxviii, 227; Study XX, Walker and Dawson: THE ARCHIVES INT. MED., 1913, xii, 171; Study XXI, Fitz: THE ARCHIVES INT. MED., 1914, xiii, 945; Study XXII, Christian: THE ARCHIVES INT. MED., 1914, xiv, 827.

1. Rowntree and Geraghty: Jour. Pharm. and Exper. Therap., 1910, i, 579; THE ARCHIVES INT. MED., 1912, ix, 284.
2. Ascoli: Pflüger's Arch. f. d. ges. Physiol., 1901, lxxxvii, 103.
3. Strauss: Die Chronische Nierentzündungen in ihrer Einwirkung auf die Blutflüssigkeit und deren Behandlung., Hirschwald, Berlin, 1902.
4. Obermayer and Popper: Ztschr. f. klin. Med., 1909, lxvii, 332.
5. Widal: Bull. et mém. Soc. méd. d. hôp., Paris, Series 3, 1911, xxxii, 627.
6. Foster: THE ARCHIVES INT. MED., 1912, x, 414.

showed that in the majority of cases the nitrogen of the blood increases with an increasingly severe nephritis, and that the degree of accumulated blood nitrogen affords valuable information in regard to the prognosis.

Heretofore, urinalysis, except in relation to the output of chlorids, nitrogen and water, has contributed little to our understanding of renal function. In 1908, however, Wohlgemuth[7] began a series of studies with the starch-splitting ferment amylase (or diastase). He found that the amount of this enzyme which was excreted in the twenty-four hour urine of normal men varied within narrow limits and was almost constant for a given individual. In the urine of nephritics, on the other hand, the amount of the enzyme excreted in the urine diminished in proportion to the severity of the disease. Wohlgemuth, therefore, suggested that the determination of amylase in urine afforded a valuable clinical test for renal function.

His results with this test have been confirmed both by experiments and by clinical studies. Hirata[8] showed that in rabbits, as well as in man, the twenty-four hour urine and the blood serum contained normally a constant amount of amylase. When corrosive sublimate, uranium nitrate, or chromic acid nephritis was produced the amylase in the urine diminished, and accumulated in the blood in almost direct proportion to the severity of the existing lesions. Von Benczur,[9] Wynhausen,[10] Rosenthal,[11] Marino,[12] Corbett[13] and Geyelin[14] have observed the excretion of amylase in nephritis and have found that it diminishes as the severity of the disease increases. Therefore there is sufficient evidence to prove that of substances constantly found in urine the amylase excretion represents a reasonably accurate test for the renal function.

The desirability of correlating as many tests for renal function as possible to determine their true values has been self-evident and has been attempted both in animal experiments and clinical studies. Frothingham, Fitz, Folin and Denis[15] produced uranium nephritis in rabbits and followed the relation between the non-protein nitrogen retention in the blood and the excretion of phenolsulphonephthalein in the urine. The two tests paralleled each other as indicators of

7. Wohlgemuth: Ueber eine neue Methode zur quantitativen Bestimmung des diastatischen Ferments.
8. Hirata: Biochem. Ztschr., 1910, xxviii, 23.
9. Von Benczur: Wien. klin. Wchnschr., 1910, xxiii, 890.
10. Wynhausen: Berl. klin. Wchnschr., 1910, xlvii, 2107.
11. Rosenthal: Deutsch. med. Wchnschr., 1911, xxxvii, 923.
12. Marino: Deutsch. Arch. f. klin. Med., 1911, ciii, 325.
13. Corbett: Quart. Jour. Med., 1913, vi, 351.
14. Geyelin: The Archives Int. Med., 1914, xiii, 96.
15. Frothingham, Fitz, Folin and Denis: The Archives Int. Med., 1913, xii, 245.

renal function, but had one essential difference. The phenolsulphonephthalein excretion in the urine dropped rapidly to its lowest point and returned rapidly to normal with recovery of the kidney. The non-protein nitrogen of the blood accumulated more gradually and returned to normal more gradually. In other words, the phenolsulphonethalein test showed the renal function at the time the test was made, while the blood nitrogen was rather a measure of an accumulating difference between the amount of waste nitrogen produced in metabolism and the amount eliminated by the kidneys. Therefore in the latter test the duration of the disease was an important factor. Confirmatory results illustrating the close parallelism of these two tests have been obtained in clinical observations by Agnew[16] and by Frothingham and Smillie.[17]

Geyelin[14] compared the excretion of amylase with that of phenolsulphonephthalein in a series of cases of nephritis and found that the results of these tests were essentially similar. Geraghty, Rowntree and Cary[18] found that although the phenolsulphonephthalein test gave more accurate information as to the degree of one-sided lesions of the kidney than the amylase test, yet the latter gave findings which were parallel.

Thus it has been shown that on the whole the excretion of amylase varies as does that of phenolsulphonephthalein in nephritis, and that as the excretion of phenolsulphonephthalein diminishes, the non-protein nitrogen of the blood accumulates. It seemed important to compare the amylase excretion in the urine and the amylase accumulation in blood serum with the accumulation of non-protein nitrogen in the blood of animals with nephritis to make comparative studies between these three tests more complete.

Accordingly, acute nephritis was produced in rabbits by means of a single dose of uranium nitrate (from 2 to 10 mg.) given subcutaneously. Two series of experiments were made. In the first series the animals were injected with uranium nitrate and were killed (under anesthesia) by bleeding from the carotid arteries. They were killed on consecutive days from one to ten days after the uranium nitrate was administered in order to secure histological record of the pathological lesions in the kidney, and to determine their relation to the non-protein nitrogen in the blood, to the amylase in the blood, and to the amylase in the urine at different stages of the nephritis. In these experiments the amylase of the urine was estimated from day to day. The blood was analyzed only on the day the experiment was begun and on the day the animals were killed. The kidney tissues were preserved for

16. Agnew: THE ARCHIVES INT. MED., 1914, xiii, 485.
17. Frothingham and Smillie: THE ARCHIVES INT. MED., 1914, xiv, 541.
18. Geraghty, Rowntree and Cary: Ann. Surg., 1913, lviii, 800.

the histological examination in Zenker's fluid and were stained with eosin and methylene blue.

In the second series of experiments, the nephritis was allowed to run its natural course. The non-protein nitrogen of the blood and the amylase of the urine were determined periodically. Amylase in the blood serum could not be estimated so often on account of the anemia induced from such frequent and relatively large bleedings.

The rabbits were kept in metabolism cages of moderate size. They were fed with carrots, oats and water. Each rabbit consumed about 100 gm. of carrot and 50 gm. of oats per day except for a few days at the height of the nephritis. In addition each rabbit was given 50 c.c. of water by stomach tube every morning to insure as constant an output of urine as possible.

The urine specimens from the metabolism cages were collected in receivers which contained 10 c.c. of toluol as a preservative. Every morning, the animals' bladders were emptied by massage and the urines so obtained were added to the containers to complete the twenty-four hour specimens.

The amylase readings in both urine and blood serum were made by the method of Wohlgemuth and Noguchi.[19] In brief, this depends on determining the amount of starch which is converted to dextrin or lower products by a known amount of urine or blood at a given temperature during a given interval of time. Iodin is used as an indicator of the starch digestion. Wohlgemuth has taken for a unit which he calls "d" the number of cubic centimeters of .1 per cent. soluble starch solution digested in one-half hour by 1 c.c. of urine or blood serum at a temperature of 38 C. "D" is the number of cubic centimeters of starch solution digested by the twenty-four hour urine. Rosenthal in obtaining the "absolute diastatic strength" of urine has preferred to divide Wohlgemuth's "D" reading by 2.

In the experiments with urine both "d" readings and the "absolute diastatic strength" were recorded to show the effect of dilution on the excretion of amylase. This question has been discussed by various observers. Wynhausen,[10] Corbett,[13] Rosenthal,[11] Von Benczur[9] and Geyelin[14] found that the influence of dilution on "d" readings was limited and without effect in the range of normal twenty-four hour urines, although allowance must be made when the quantities were abnormally large or small. In the experiments reported, such marked variations in urinary output occurred from day to day that the necessity of considering the effect of dilution was obvious.

19. Wolgemuth and Noguchi: Berl. klin. Wchnschr., 1912, xlix, 1096. This method is described in detail by Geyelin (Note 14) and therefore the description is not repeated here.

In the experiments with blood, 5 c.c. were withdrawn and defibrinated by shaking with beads. The serum was obtained by centrifugalization, and its "d" reading made by the method mentioned. Folin and Denis'[20] micromethod on oxalated blood was used for the blood nitrogen determinations.

EXPERIMENTS

Experiment 1.—Rabbit killed on the *first day* after the administration of the uranium nitrate.

First day. (Wt. 1,650 gm.)		
Urine:	twenty-four-hour amount	150 c.c.
	"d"	32
	absolute diastatic strength	2,400
Blood:	serum "d"	32
	non-protein nitrogen (per 100 gm.)	37 mg.
Administered 3 mg. uranium nitrate.		
Second day. (Wt. 1,700 gm.)		
Urine:	twenty-four-hour amount	150 c.c.
	"d"	32
	absolute diastatic strength	2,400
Blood:	serum "d"	32
	non-protein nitrogen (per 100 gm.)	54

Histological findings: Glomeruli negative; tubules negative. No pathological changes found in the kidney.

Experiment 2.—Rabbit killed on the *second day* after the administration of the uranium nitrate.

First day. (Wt. 1,600 gm.)		
Urine:	twenty-four-hour amount	150 c.c.
	"d"	32
	absolute diastatic strength	2,400
Blood:	serum "d"	64
	non-protein nitrogen (per 100 gm.)	25 mg.
Administered 6 mg. uranium nitrate.		
Second day. (Wt. 1,700 gm.)		
Urine:	twenty-four-hour amount	180 c.c.
	"d"	32
	absolute diastatic strength	2,880
Third day. (Wt. 1,700 gm.)		
Urine:	twenty-four-hour amount	150 c.c.
	"d"	64
	absolute diastatic strength	4,800
Blood:	serum "d"	64
	non-protein nitrogen (per 100 gm.)	44 mg.

Histological Findings: Glomeruli negative; slight necrosis and desquamation of the epithelium of certain tubules; a few tubules filled with necrotic material; a few areas of round cell infiltration.

Experiment 3.—Rabbit killed on the *third day* after the administration of the uranium nitrate.

First day. (Wt. 2,600 gm.)		
Urine:	twenty-four-hour amount	150 c.c.
	"d"	128
	absolute diastatic strength	9,600
Blood:	serum "d"	64
	non-protein nitrogen (per 100 gm.)	37 mg.
Administered 6 mg. uranium nitrate.		

20. Folin and Denis: Jour. Biol. Chem., 1912, xi, 257.

Second day. (Wt. 2,600 gm.)
Urine: twenty-four-hour amount ..... 160 c.c.
"d" 128
absolute diastatic strength 10,240
Third day. (Wt. 2,500 gm.)
Urine: twenty-four-hour amount ..... 80 c.c.
"d" 128
absolute diastatic strength 5,120
Fourth day. (Wt. 2,450 gm.)
Urine: twenty-four-hour amount ..... 7 c.c.
"d" 64
absolute diastatic strength 224
Blood: serum "d" 128
non-protein nitrogen (per 100 gm.)..... 131 mg.

Histological Findings: Glomeruli negative. Considerable necrosis and desquamation of tubular epithelium; many tubules filled with necrotic epithelium and casts; many tubules lined with regenerating epithelium.

Experiment 4.—Rabbit killed on the *fourth day* after the administration of uranium nitrate.

First day. (Wt. 2,150 gm.)
Urine: twenty-four-hour amount..... 110 c.c.
"d" 128
absolute diastatic strength 7,040
Blood: serum "d" 64
non-protein nitrogen (per 100 gm.)..... 30 mg.
Administered 7 mg. uranium nitrate.
Second day. (Wt. 2,050 gm.)
Urine: twenty-four-hour amount ..... 100 c.c.
"d" 128
absolute diastatic strength 6,400
Third day. (Wt. 2,000 gm.)
Urine: twenty-four-hour amount ..... 90 c.c.
"d" 128
absolute diastatic strength 5,760
Fourth day. (Wt. 1,930 gm.)
Urine: twenty-four-hour amount ..... 8 c.c.
"d" 32
absolute diastatic strength 128
Fifth day. (Wt. 1,950 gm.)
Urine: twenty-four-hour amount ..... Anuric
"d" ....
absolute diastatic strength..... ...
Blood: serum "d" 64
non-protein nitrogen (per 100 gm.) ..... 196 mg.

Histological Findings: Glomeruli negative; considerable necrosis and desquamation of the epithelium of many tubules; many tubules filled with necrotic material, a few with casts; a few tubules lined with regenerating epithelium.

Experiment 5.—Rabbit killed on the *fifth day* after the administration of the uranium nitrate.

First day. (Wt. 2,050 gm.)
Urine: twenty-four-hour amount ..... 180 c.c.
"d" 32
absolute diastatic strength 2,880
Blood: serum "d" 32
non-protein nitrogen (per 100 gm.)..... 31 mg.
Administered 5 mg. uranium nitrate.
Second day. (Wt. 2,000 gm.)
Urine: twenty-four-hour amount ..... 170 c.c.
"d" 32
absolute diastatic strength 2,720

Third day. (Wt. 1,900 gm.)
Urine: twenty-four-hour amount ..... 100 c.c.
"d" 32
absolute diastatic strength 1,600
Fourth day. (Wt. 1,900 gm.)
Urine: twenty-four-hour amount ..... 75 c.c.
"d" 32
absolute diastatic strength 1,200
Fifth day. (Wt. 1,850 gm.)
Urine: twenty-four-hour amount ..... 80 c.c.
"d" 32
absolute diastatic strength 1,280
Sixth day. (Wt. 1,850 gm.)
Urine: twenty-four-hour amount ..... 110 c.c.
"d" 16
absolute diastatic strength 880
Blood: serum "d" 64
non-protein nitrogen (per 100 gm.)..... 78 mg.

Histological Findings: Glomeruli negative; considerable necrosis and desquamation of the epithelium of many tubules; a few tubules filled with necrotic material; many tubules lined with regenerating epithelium.

Experiment 6.—Rabbit killed on the *sixth day* after the administration of the uranium nitrate.

First day. (Wt. 2,100 gm.)
Urine: twenty-four-hour amount ..... 80 c.c.
"d" 16
absolute diastatic strength 640
Blood: serum "d" 64
non-protein nitrogen (per 100 gm.)..... 35 mg.
Administered 7 mg. uranium nitrate.
Second day. (Wt. 2,100 gm.)
Urine: twenty-four-hour amount ..... 65 c.c.
"d" 64
absolute diastatic strength..... 2,080
Third day. (Wt. 2,100 gm.)
Urine: twenty-four-hour amount ..... 100 c.c.
"d" 64
absolute diastatic strength 3,200
Fourth day. (Wt. 2,000 gm.)
Urine: twenty-four-hour amount ..... 60 c.c.
"d" 64
absolute diastatic strength..... 1,920
Fifth day. (Wt. 2,000 gm.)
Urine: twenty-four-hour amount ..... 10 c.c.
"d" 4
absolute diastatic strength 20
Sixth day. (Wt. 2,000 gm.)
Urine: twenty-four-hour amount ..... 20 c.c.
"d" 8
absolute diastatic strength 80
Seventh day. (Wt. 2,000 gm.)
Urine: twenty-four-hour amount ..... 30 c.c.
"d" 4
absolute diastatic strength 60
Blood: serum "d" 128
non-protein nitrogen (per 100 gm.)..... 217 mg.

Histological findings: Hyaline droplets in many glomeruli; considerable necrosis and desquamation of the epithelium of many tubules; many tubules filled with necrotic material and casts; many tubules lined with regenerating epithelium.

Experiment 7.—Rabbit killed on the *seventh day* after the administration of the uranium nitrate.

First day. (Wt. 2,300 gm.)		
Urine:	twenty-four-hour amount	130 c.c.
	"d"	16
	absolute diastatic strength	1,040
Blood:	serum "d"	32
	non-protein nitrogen (per 100 gm.)	38 mg.
Administered 7 mg. uranium nitrate.		
Second day. (Wt. 2,250 gm.)		
Urine:	twenty-four-hour amount	15 c.c.
	"d"	32
	absolute diastatic strength	240
Third day. (Wt. 2,200 gm.)		
Urine:	twenty-four-hour amount	200 c.c.
	"d"	32
	absolute diastatic strength	3,200
Fourth day: (Wt. 2,150 gm.)		
Urine:	twenty-four-hour amount	130 c.c.
	"d"	16
	absolute diastatic strength	1,040
Fifth day. (Wt. 1,950 gm.)		
Urine:	twenty-four-hour amount	70
	"d"	16
	absolute diastatic strength	560
Sixth day. (Wt. 2,000 gm.)		
Urine:	twenty-four-hour amount	100 c.c.
	"d"	16
	absolute diastatic strength	800
Seventh day. (Wt. 1,900 gm.)		
Urine:	twenty-four-hour amount	170 c.c.
	"d"	12
	absolute diastatic strength	1,020
Eighth day. (Wt. 1,700 gm.)		
Urine:	twenty-four-hour amount	120 c.c.
	"d"	16
	absolute diastatic strength	960
Blood:	Serum "d"	128
	non-protein nitrogen (per 100 gm.)	250 mg.

Histological Findings: Glomeruli negative; desquamation of the epithelium of a few tubules; many tubules filled with serum and casts; many tubules lined with regenerating epithelium; slight increase in connective tissue throughout the kidney, suggesting preexisting chronic nephritis.

Experiment 8.—Rabbit killed on the *eighth day* after the administration of the uranium nitrate.

First day. (Wt. 1,500 gm.)		
Urine:	twenty-four-hour amount	140 c.c.
	"d"	32
	absolute diastatic strength	2,240
Blood:	serum "d"	64
	non-protein nitrogen (per 100 gm.)	27 mg.
Administered 4 mg. uranium nitrate.		
Second day. (Wt. 1,450 gm.)		
Urine:	twenty-four-hour amount	150 c.c.
	"d"	32
	absolute diastatic strength	2,400
Third day. (Wt. 1,500 gm.)		
Urine:	twenty-four-hour amount	110 c.c.
	"d"	32
	absolute diastatic strength	1,760

Fourth day. (Wt. 1,500 gm.)		
Urine: twenty-four-hour amount	170	c.c.
"d"	16	
absolute diastatic strength	1,360	
Fifth day. (Wt. 1,450 gm.)		
Urine: twenty-four-hour amount	120	c.c.
"d"	16	
absolute diastatic strength	960	
Sixth day. (Wt. 1,450 gm.)		
Urine: twenty-four-hour amount	120	c.c.
"d"	16	
absolute diastatic strength	960	
Seventh day. (Wt. 1,400 gm.)		
Urine: twenty-four-hour amount	120	c.c.
"d"	16	
absolute diastatic strength	960	
Eighth day. (Wt. 1,350 gm.)		
Urine: twenty-four-hour amount	130	c.c.
"d"	16	
absolute diastatic strength	1,040	
Ninth day. (Wt. 1,300 gm.)		
Urine: twenty-four-hour amount	75	c.c.
"d"	32	
absolute diastatic strength	1,200	
Blood: serum "d"	64	
non-protein nitrogen (per 100 gm.)	83	mg.

Histological Findings: Glomeruli negative; desquamation of the epithelium of a few tubules; many tubules filled with casts and a few with necrotic cells; a few tubules lined with regenerating epithelium.

Experiment 9.—Rabbit killed on the *ninth day* after the administration of uranium nitrate.

First day. (Wt. 2,000 gm.)		
Urine: twenty-four-hour amount	180	c.c.
"d"	32	
absolute diastatic strength	2,880	
Blood: serum "d"	32	
non-protein nitrogen (per 100 gm.)	39	mg.
Administered 4 mg. uranium nitrate.		
Second day. (Wt. 2,100 gm.)		
Urine: twenty-four-hour amount	140	c.c.
"d"	64	
absolute diastatic strength	4,680	
Third day. (Wt. 2,150 gm.)		
Urine: twenty-four-hour amount	150	c.c.
"d"	32	
absolute diastatic strength	2,400	
Fourth day. (Wt. 2,100 gm.)		
Urine: twenty-four-hour amount	230	c.c.
"d"	32	
absolute diastatic strength	3,680	
Fifth day. (Wt. 2,100 gm.)*		
Urine: twenty-four-hour amount	210	c.c.
"d"	32	
absolute diastatic strength	3,360	
Sixth day. (Wt. 2,050 gm.)		
Urine: twenty-four-hour amount	180	c.c.
"d"	64	
absolute diastatic strength	5,760	

*On the fifth day the animal received an additional injection of 7 mg. of uranium nitrate.

Seventh day. (Wt. 2,050 gm.)
Urine: twenty-four-hour amount..... 150 c.c.
"d" 64
absolute diastatic strength..... 4,800
Eighth day. (Wt. 1,950 gm.)
Urine: twenty-four-hour amount..... 100 c.c.
"d" 32
absolute diastatic strength..... 1,600
Ninth day. (Wt. 1,950 gm.)
Urine: twenty-four-hour amount..... 90 c.c.
"d" 32
absolute diastatic strength..... 1,440
Tenth day. (Wt. 1,900 gm.)
Urine: twenty-four-hour amount..... 100 c.c.
"d" 32
absolute diastatic strength..... 1,600
Blood: serum "d"..... 64
non-protein nitrogen (per 100 gm.)..... 96 mg.

Histological Findings: Glomeruli negative; a few tubules lined with regenerating epithelium; many tubules filled with casts.

Experiment 10.—Rabbit killed on the tenth day after the administration of uranium nitrate.

First day. (Wt. 2,300 gm.)
Urine: twenty-four-hour amount..... 180 c.c.
"d" 64
absolute diastatic strength..... 5,760
Blood: serum "d" 64
non-protein nitrogen (per 100 gm.)..... 30 mg.
Administered 6 mg. uranium nitrate.
Second day. (Wt. 2,300 gm.)
Urine: twenty-four-hour amount..... 120 c.c.
"d" 64
absolute diastatic strength..... 3,840
Third day. (Wt. 2,300 gm.)
Urine: twenty-four-hour amount..... 180 c.c.
"d" 64
absolute diastatic strength..... 5,760
Fourth day. (Wt. 2,350 gm.)
Urine: twenty-four-hour amount..... 90 c.c.
"d" 64
absolute diastatic strength..... 2,850
Fifth day. (Wt. 2,250 gm.)
Urine: twenty-four-hour amount..... 170 c.c.
"d" 32
absolute diastatic strength..... 2,720
Sixth day. (Wt. 2,200 gm.)
Urine: twenty-four-hour amount..... 210 c.c.
"d" 32
absolute diastatic strength..... 3,360
Seventh day. (Wt. 2,150 gm.)
Urine: twenty-four-hour amount..... 170 c.c.
"d" 64
absolute diastatic strength..... 5,440
Eighth day. (Wt. 2,150 gm.)
Urine: twenty-four-hour amount..... 220 c.c.
"d" 50
absolute diastatic strength..... 5,500
Ninth day. (Wt. 2,150 gm.)
Urine: twenty-four-hour amount..... 190 c.c.
"d" 64
absolute diastatic strength..... 6,080

Tenth day. (Wt. 2,200 gm.)		
Urine: twenty-four-hour amount	170	c.c.
"d"	64	
absolute diastatic strength	5,440	
Eleventh day. (Wt. 2,250 gm.)		
Urine: twenty-four-hour amount	170	c.c.
"d"	64	
absolute diastatic strength	5,440	
Blood: serum "d"	64	
non-protein nitrogen (per 100 gm.)	24	mg.

Histological Findings: Glomeruli negative; a few tubules filled with casts.

From Experiments 1 to 10 recorded above, it is seen that the findings in regard to the non-protein nitrogen of the blood confirm those recorded by Frothingham, Fitz, Folin and Denis.[15] The accumulation of nitrogen was gradual. It became high after the third day, remained at a high level from the third to the eighth day, and then gradually returned to normal. When extensive necrosis of the renal epithelium occurred, the blood nitrogen accumulated rapidly, and diminished slowly with repair of the kidney. Thus this test paralleled in a general way the anatomical damage in the kidney.

The amylase readings in the urine varied considerably in animals supposed to be normal. The "d" readings ran from 16 to 128 units, and the "absolute diastatic strength" from 640 to 5,760 units. This may in part be due to the fact that the animals were not kept under a longer period of preliminary observation. They were taken from stock and injected with uranium nitrate after one twenty-four hour specimen of urine was collected. Nevertheless the blood nitrogen and the blood "d" readings were much more constant under the same conditions.

In six of the ten experiments there was an increase in the "absolute diastatic strength" of the urine, and in two experiments of the "d" reading, following the injection of the uranium nitrate. This lasted for a short time. It was not caused by an increased output of urine in every case, and suggested a stimulated renal function which was not confirmed, however, by a diminished blood nitrogen.

The "absolute diastatic strength" and the "d" of the urine diminished in each case as the nephritis became severe. Both readings paralleled roughly the severity of the lesion as judged by anatomical changes in the kidney. The "absolute diastatic strength" seemed to show finer distinctions in regard to the renal function than did the "d" readings. This was largely because of the variations in the twenty-four hour urine which occurred from day to day.

The blood "d" readings did not increase as much as would be presumed from the variations in the excretion of amylase. In four cases there was a definite rise in "d" at the end of the experiment when the nephritis was most severe. It did not parallel the accumulation of blood nitrogen, nor accompany an extremely low amylase excretion in

each case and was not sufficiently marked to make the determinations of particular diagnostic or prognostic value.

Thus on the whole, in the individual cases there was a definite relation between the nephritis, the accumulation of non-protein nitrogen in the blood, and the excretion of amylase in the urine. The blood "d" readings were insignificant.

In order to compare more accurately the relation of the accumulation of the non-protein nitrogen in the blood with the excretion of amylase at different stages of the acute nephritis, a second series of experiments was made. In this series the blood was collected every day from the ear veins and the amylase excretion was followed. Experiments 11 to 19 recorded below, give the important points of these observations.

Experiment 11.—Rabbit killed on the *fourth day* after the administration of uranium nitrate.

First day. (Wt. 1,700 gm.)		
Urine: twenty-four-hour amount	143	c.c.
"d"	128	
absolute diastatic strength	9,152	
Blood: non-protein nitrogen (per 100 gm.)	24	mg.
Administered 3 mg. uranium nitrate.		
Third day. (Wt. 1,800 gm.)		
Urine: twenty-four-hour amount	120	c.c.
"d"	64	
absolute diastatic strength	3,840	
Blood: non-protein nitrogen (per 100 gm.)	30	mg.
Fifth day. (Wt. 1,550 gm.)		
Urine: twenty-four-hour amount	15	c.c.
absolute diastatic strength	120	
Blood: non-protein nitrogen (per 100 gm.)	125	mg.

Histological Findings: Glomeruli negative; considerable necrosis and desquamation of the epithelium of certain tubules; many other tubules filled with necrotic material and casts; a few other tubules lined with regenerated epithelium; a few areas of round-cell infiltration.

Experiment 12.—Rabbit found dead on *sixth day* after the administration of uranium nitrate.

First day. (Wt. 2,500 gm.)		
Urine: twenty-four-hour amount	105	c.c.
"d"	256	
absolute diastatic strength	13,440	
Blood: non-protein nitrogen (per 100 gm.)	28	mg.
Administered 4 mg. uranium nitrate.		
Fourth day. (Wt. 2,550 gm.)		
Urine: twenty-four-hour amount	135	c.c.
"d"	64	
absolute diastatic strength	4,320	
Blood: non-protein nitrogen (per 100 gm.)	51	mg.
Fifth day. (Wt. 2,500 gm.)		
Urine: twenty-four-hour amount	60	c.c.
"d"	128	
absolute diastatic strength	3,840	
Blood: non-protein nitrogen (per 100 gm.)	80	mg.

Sixth day. (Wt. 2,500 gm.)
Urine: twenty-four-hour amount ..... 10 c.c.
"d" 128
absolute diastatic strength..... 640
Blood: non-protein nitrogen (per 100 gm.)..... 125 mg.

Histological Findings: Glomeruli congested; considerable necrosis and desquamation of the epithelium of certain tubules; many other tubules filled with necrotic material and casts; a few other tubules lined with regenerated epithelium.

Experiment 13.—Rabbit found dead on sixth day after the administration of uranium nitrate.

First day. (Wt. 2,300 gm.)
Urine: twenty-four-hour amount..... 100 c.c.
"d" 64
absolute diastatic strength..... 3,200
Blood: non-protein nitrogen (per 100 gm.)..... 25 mg.
Administered 8 mg. uranium nitrate.
Third day. (Wt. 2,350 gm.)
Urine: twenty-four-hour amount..... 40 c.c.
"d" 128
absolute diastatic strength..... 2,560
Blood: non-protein nitrogen (per 100 gm.)..... 41 mg.
Fourth day. (Wt. 2,350 gm.)
Urine: twenty-four-hour amount ..... 3 c.c.
"d" 32
absolute diastatic strength..... 48
Blood: non-protein nitrogen (per 100 gm.)..... 70 mg.
Fifth day. (Wt. 2,300 gm.)
Urine: twenty-four-hour amount..... 5 c.c.
"d" 2
absolute diastatic strength..... 5
Blood: non-protein nitrogen (per 100 gm.)..... 96 mg.
Sixth day. (Wt. 2,300 gm.)
Urine: twenty-four-hour amount..... 7 c.c.
"d" 16
absolute diastatic strength..... 56
Blood: non-protein nitrogen (per 100 gm.)..... 125 mg.

Histological Findings: Glomeruli negative: necrosis and desquamation of the epithelium of certain tubules; few tubules filled with necrotic material, but many filled with casts; many tubules lined with regenerated epithelium.

Experiment 14.—Rabbit killed on the seventh day after the administration of uranium nitrate.

First day. (Wt. 2,000 gm.)
Urine: twenty-four-hour amount..... 120 c.c.
"d" 128
absolute diastatic strength..... 7,680
Blood: non-protein nitrogen (per 100 gm.)..... 30 mg.
Administered 2.5 mg. uranium nitrate.
Second day. (Wt. 1,950 gm.)
Urine: twenty-four-hour amount..... 170 c.c.
"d" 128
absolute diastatic strength..... 10,880
Blood: non-protein nitrogen (per 100 gm.)..... 35 mg.
Fourth day. (Wt. 1,950 gm.)
Urine: twenty-four-hour amount..... 17 c.c.
"d" 256
absolute diastatic strength..... 2,176
Blood: non-protein nitrogen (per 100 gm.)..... 136 mg.

Sixth day. (Wt. 1,950 gm.)
Urine: twenty-four-hour amount..... 20 c.c.
"d" 4
absolute diastatic strength..... 40
Blood: non-protein nitrogen (per 100 gm.)..... 200 mg.
Seventh day. (Wt. 1,950 gm.)
Urine: twenty-four-hour amount..... 8 c.c.
"d" 8
absolute diastatic strength..... 32
Blood: non-protein nitrogen (per 100 gm.)..... 238 mg.

Histological Findings: Glomeruli negative; considerable necrosis and desquamation of the epithelium of certain tubules; many tubules filled with necrotic material and casts; many tubules lined with regenerated epithelium.

Experiment 15.—Experiment discontinued on the ninth day after the administration of the uranium nitrate.

First day. (Wt. 2,700 gm.)
Urine: twenty-four-hour amount..... 150 c.c.
"d" 128
absolute diastatic strength..... 9,600
Blood: non-protein nitrogen (per 100 gm.)..... 27 mg.
Administered 4 mg. uranium nitrate.
Third day. (Wt. 2,650 gm.)
Urine: twenty-four-hour amount..... 200 c.c.
"d" 64
absolute diastatic strength..... 6,400
Blood: non-protein nitrogen (per 100 gm.)..... 37 mg.
Fifth day. (Wt. 2,600 gm.)
Urine: twenty-four-hour amount..... 180 c.c.
"d" 64
absolute diastatic strength..... 5,760
Blood: non-protein nitrogen (per 100 gm.)..... 71 mg.
Seventh day. (Wt. 2,450 gm.)
Urine: twenty-four-hour amount..... 100 c.c.
"d" 200
absolute diastatic strength..... 10,000
Blood: non-protein nitrogen (per 100 gm.)..... 54 mg.
Ninth day. (Wt. 2,350 gm.)
Urine: twenty-four-hour amount..... 100 c.c.
"d" 128
absolute diastatic strength..... 6,400
Blood: non-protein nitrogen (per 100 gm.)..... 43 mg.

Experiment 16.—Rabbit killed on the tenth day after the administration of uranium nitrate.

First day. (Wt. 2,700 gm.)
Urine: twenty-four-hour amount..... 220 c.c.
"d" 64
absolute diastatic strength..... 7,040
Blood: non-protein nitrogen (per 100 gm.)..... 38 mg.
Administered 4 mg. uranium nitrate.
Third day. (Wt. 2,700 gm.)
Urine: twenty-four-hour amount..... 220 c.c.
"d" 128
absolute diastatic strength..... 14,080
Blood: non-protein nitrogen (per 100 gm.)..... 46 mg.
Fifth day. (Wt. 2,600 gm.)
Urine: twenty-four-hour amount..... 50 c.c.
"d" 64
absolute diastatic strength..... 1,600
Blood: non-protein nitrogen (per 100 gm.)..... 102 mg.

Sixth day. (Wt. 2,500 gm.)
Urine: twenty-four-hour amount..... 70 c.c.
"d" 24
absolute diastatic strength..... 840
Blood: non-protein nitrogen (per 100 gm.)..... 161 mg.
Eighth day. (Wt. 2,300 gm.)
Urine: twenty-four-hour amount..... 80 c.c.
"d" 32
absolute diastatic strength..... 1,280
Blood: non-protein nitrogen (per 100 gm.)..... 208 mg.
Tenth day. (Wt. 2,100 gm.)
Urine: twenty-four-hour amount..... 140 c.c.
"d" 64
absolute diastatic strength..... 4,680
Blood: non-protein nitrogen (per 100 gm.)..... 165 mg.

Histological Findings: Glomeruli negative; epithelium of tubules negative; many tubules contain hyaline casts.

Experiment 17.—Rabbit found dead on the tenth day after the administration of the uranium nitrate.

First day. (Wt. 1,900 gm.)
Urine: twenty-four-hour amount..... 100 c.c.
"d" 128
absolute diastatic strength..... 6,400
Blood: non-protein nitrogen (per 100 gm.)..... 34 mg.
Administered 2 mg. uranium nitrate.
Third day. (Wt. 2,050 gm.)
Urine: twenty-four-hour amount..... 95 c.c.
"d" 64
absolute diastatic strength..... 3,040
Blood: non-protein nitrogen (per 100 gm.)..... 41 mg.
Fifth day. (Wt. 2,050 gm.)
Urine: twenty-four-hour amount..... 45 c.c.
"d" 32
absolute diastatic strength..... 720
Blood: non-protein nitrogen (per 100 gm.)..... 113 mg.
Seventh day. (Wt. 1,900 gm.)
Urine: twenty-four-hour amount..... 90 c.c.
"d" 32
absolute diastatic strength..... 1,440
Blood: non-protein nitrogen (per 100 gm.)..... 208 mg.
Eighth day. (Wt. 1,800 gm.)
Urine: twenty-four-hour amount..... 185 c.c.
"d" 32
absolute diastatic strength..... 2,960
Blood: non-protein nitrogen (per 100 gm.)..... 250 mg.
Tenth day. (Wt. 1,550 gm.)
Urine: twenty-four-hour amount..... 200 c.c.
"d" 32
absolute diastatic strength..... 3,200
Blood: non-protein nitrogen (100 per gm.)..... 151 mg.

Histological Findings: Glomeruli negative. A few tubules filled with necrotic material; many tubules filled with hyaline casts; a few tubules lined with regenerating epithelium. A few areas of round-cell infiltration.

Experiment 18.—Experiment discontinued on the thirteenth day after the administration of uranium nitrate.

First day. (Wt. 2,200 gm.)
Urine: twenty-four-hour amount..... 160 c.c.
"d" 128
absolute diastatic strength..... 10,240
Blood: non-protein nitrogen (per 100 gm.)..... 30 mg.
Administered 2.5 mg. uranium nitrate.

Second day. (Wt. 2,200 gm.)
Urine: twenty-four-hour amount..... 150 c.c.
"d" 128
absolute diastatic strength..... 9,600
Blood: non-protein nitrogen (per 100 gm.)..... 39 mg.
Fourth day. (Wt. 2,100 gm.)
Urine: twenty-four-hour amount..... 125 c.c.
"d" 32
absolute diastatic strength..... 2,000
Blood: non-protein nitrogen (per 100 gm.)..... 83 mg.
Sixth day. (Wt. 2,000 gm.)
Urine: twenty-four-hour amount..... 155 c.c.
"d" 32
absolute diastatic strength..... 2,480
Blood: non-protein nitrogen (per 100 gm.)..... 104 mg.
Eighth day. (Wt. 1,900 gm.)
Urine: twenty-four-hour amount..... 170 c.c.
"d" 64
absolute diastatic strength..... 5,440
Blood: non-protein nitrogen (per 100 gm.)..... 96 mg.
Tenth day. (Wt. 1,950 gm.)
Urine: twenty-four-hour amount..... 170 c.c.
"d" 64
absolute diastatic strength..... 5,440
Blood: non-protein nitrogen (per 100 gm.)..... 83 mg.
Eleventh day. (Wt. 1,850 gm.)
Urine: twenty-four-hour amount..... 210 c.c.
"d" 64
absolute diastatic strength..... 6,720
Blood: non-protein nitrogen (per 100 gm.)..... 52 mg.
Thirteenth day. (Wt. 2,000 gm.)
Urine: twenty-four-hour amount..... 110 c.c.
"d" 128
absolute diastatic strength..... 7,040
Blood: non-protein nitrogen (per 100 gm.)..... 27 mg.

Experiment 19.—Experiment discontinued on the fifteenth day after the injection of uranium nitrate.

First day. (Wt. 2,500 gm.)
Urine: twenty-four-hour amount..... 200 c.c.
"d" 100
absolute diastatic strength..... 10,000
Blood: non-protein nitrogen (per 100 gm.)..... 29 mg.
Administered 6 mg. uranium nitrate.
Second day. (Wt. 2,400 gm.)
Urine: twenty-four-hour amount..... 170 c.c.
"d" 100
absolute diastatic strength..... 8,500
Blood: non-protein nitrogen (per 100 gm.)..... 43 mg.
Fifth day. (Wt. 2,350 gm.)
Urine: twenty-four-hour amount..... 200 c.c.
"d" 12
absolute diastatic strength..... 1,200
Blood: non-protein nitrogen (per 100 gm.)..... 54 mg.
Seventh day. (Wt. 2,400 gm.)
Urine: twenty-four-hour amount..... 150 c.c.
"d" 16
absolute diastatic strength..... 1,200
Blood: non-protein nitrogen (per 100 gm.)..... 74 mg.
Tenth day. (Wt. 2,100 gm.)
Blood: non-protein nitrogen (per 100 gm.)..... 78 mg.

Thirteenth day. (Wt. 2,200 gm.)		
Urine: twenty-four-hour amount	150	c.c.
"d"	32	
absolute diastatic strength	2,400	
Blood: non-protein nitrogen (per 100 gm.)	40	mg.
Fifteenth day. (Wt. 2,400 gm.)		
Urine: twenty-four-hour amount	170	c.c.
"d"	64	
absolute diastatic strength	5,440	
Blood: non-protein nitrogen (per 100 gm.)	31	mg.

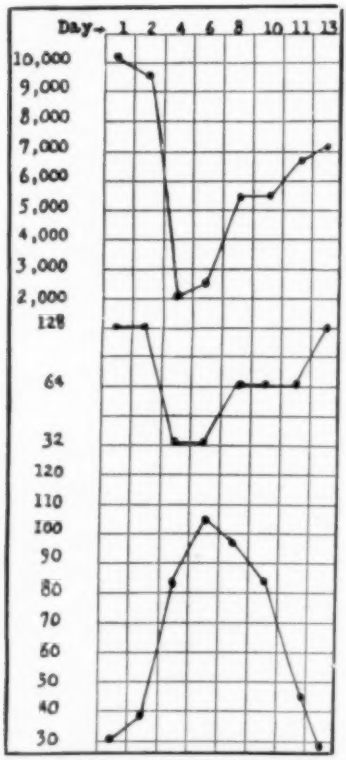

Chart 1.—Curves showing "absolute diastatic" strength (upper curve), "d" excretion (middle curve), and (lower curve) non-protein nitrogen retention in a moderate nephritis (Exp. 18).

The correspondence between the results of the amylase tests and those of the blood analyses in Experiments 11 to 19 is shown graphically in the accompanying charts, (a) with reference to a moderate degree of nephritis (Exp. 18), (b) with reference to severe nephritis (Exp. 17).

From the tables and charts it is clear that at the beginning of the nephritis the "absolute diastatic strength" of the urine dropped more rapidly than the accumulation of the non-protein nitrogen of the blood. During the course of the disease the height of the nitrogenous accumu-

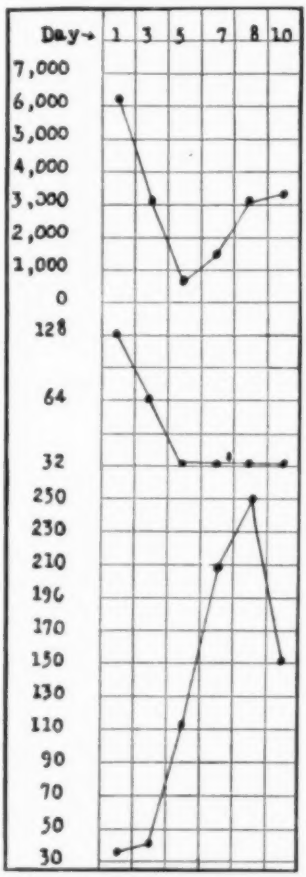

Chart 2.—Curves showing "absolute diastatic" strength (upper curve), "d" excretion (middle curve), and (lower curve) non-protein nitrogen retention in a severe nephritis (Exp. 17).

lation was reached later than the lowest level of the amylase excretion. After the kidney function had begun to improve as shown by an increasing elimination of amylase, the retention nitrogen did not at once begin to recede but even continued to rise for a short period.

Thus the amylase test is very similar to the phenolsulphonephthalein test. It has certain disadvantages. In a series of nineteen rabbits, the normal excretion showed such marked variation that the information obtained from the test could only be applied to the individual case. Therefore in studying the renal function of any one it was first necessary to discover the normal excretion for that animal. It is only fair to state, however, that the results obtained in man by other observers tend to show that there is much less normal individual variation than in the experiments reported here. A second disadvantage with the test is that it is less quantitative than the phenolsulphonephthalein test. If the "absolute diastatic strength" is taken, small changes in the output of urine may exaggerate changes in renal function. If the "d" reading is accepted, slight changes in renal function are overlooked. Possibly a different method of diluting the urine, as suggested by Brown and Smith,[21] may obviate this source of error.

On the whole, these experiments show that there is a close parallelism between the modes of excretion of phenolsulphonephthalein and amylase, and that amylase like phenolsulphonephthalein diminishes with an increasing nephritis, returns to normal with improvement of renal function, and is not influenced by blood retention to the same extent as is the accumulation of non-protein nitrogen.

CONCLUSIONS

1. In acute uranium nephritis in rabbits, the excretion of amylase in the urine and the amount of non-protein nitrogen in the blood vary from the normal during the course of the nephritis and return to normal as the nephritis heals.

2. In individual cases the degree of variation from the normal agrees on the whole with the amount of destruction demonstrated histologically in the kidney.

3. The amylase excretion, like that of phenolsulphonephthalein, drops rapidly to its lowest point and returns rapidly toward its previous level with recovery of the kidney. It is but little influenced by accumulation in the blood.

4. The non-protein nitrogen accumulates gradually in the blood, and returns to normal gradually as the kidney recovers.

5. Amylase excretion as a test for renal function is similar to phenolsulphonephthalein, but is less delicate.

6. The amylase and phenolsulphonephthalein tests show the renal function at the moment; the blood nitrogen test is more influenced by the duration of the condition causing its accumulation.

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21. Brown and Smith: Bull. Johns Hopkins Hosp., 1914, xxv, 213.

FURTHER STUDIES OF RENAL FUNCTION IN RENAL, CARDIORENAL AND CARDIAC DISEASES *

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The objects of this investigation are (1) to ascertain the value of quantitative determinations of the diastatic activity[1] of urine in revealing renal functional capacity in cardiac, cardiorenal and renal cases; (2) to compare in the same group of cases the findings of diastase, urea and phthalein in the urine with those of urea, total incoagulable nitrogen and cryoscopy in the blood, and (3) to compare the relative value and limitations of the tests of retention with those of excretion.

Renal functional capacity is usually ascertained in one of two ways: (1) *Tests of excretory capacity* through the quantitative determination of the secretion of various substances in the urine — dyes, methylene blue, indigocarmin, rosanilin and phthalein; other chemicals, potassium iodid, lactose, salicylates, sodium chlorid, urea, sugar following phloridzin and the enzyme, diastase. (2) *Tests of retention* through the determination of the concentration of certain substances in the blood, ions — through electrical conductivity, molecules and ions — through cryoscopy, and urea, total incoagulable nitrogen and cholesterin.

The number of functional tests has increased to such an extent that it is essential to determine which can be discarded without loss. Only through familiarity with the reliability, value, limitations, peculiarities, and the significance of the findings of each test in the various types of disease is the most profitable selection of tests made possible.

THE TESTS EMPLOYED AND THEIR TECHNIC

1. The *phthalein* test[2] was used according to the usual technic.

2. *Diastase.*[3] Diastase has recently been introduced into functional renal work by Wohlgemuth[4] for determining the relative functional

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1. The expression "diastatic activity of the urine" is used in place of "diastase content" or "quantitative urinary diastase," because at present we have no method for the quantitative estimation of an enzyme, but only methods for the quantitative expression of relative enzymatic activity.

2. Rowntree and Geraghty: THE ARCHIVES INT. MED., 1912, ix, 284.

3. For the results of our diastase studies in unilateral and bilateral surgical diseases of the kidneys, see Ann. Surg., 1913, xxxvii, 801; Surg., Gynec. and Obst., 1914, xviii, 196.

4. Wohlgemuth: Biol. Ztschr., 1909, xxi, 432.

capacity of the two kidneys. A modification of this method was devised by us to make the test adaptable for determining total renal functional capacity. The technic employed was elaborated in conjunction with Dr. Thomas R. Brown, who has demonstrated with it that the total daily output in urine is fairly constant.[5] The technic was as follows:

One-fifth of the twenty-four-hour urine collected under toluol was neutralized and diluted to 1 L. By means of a graduated 2 c.c. pipet, decreasing amounts of urine were placed in a series of twelve tubes (arranged in a rack) as follows: 1.8, 1.6, 1.4, 1.2, 1, 0.8, 0.6, 0.4 c.c. Ten c.c. of the diluted urine was further diluted to 20 c.c. and of this decreasing amounts placed in the remaining tubes of the series as follows: 0.4, 0.2, 0.1, 0.05 c.c. To each tube was added sufficient 1 per cent. NaCl solution to bring the total volume in each tube to 2 c.c. Two c.c. of a 0.1 per cent. freshly prepared soluble starch solution was added to each tube. The rack with the tubes was placed in a water-bath at 38 C. for a half-hour, then transferred to cold water for three minutes. $\frac{N}{50}$ iodin solution was added drop by drop in amounts sufficient to elicit a permanent color. The occurrence of blue or violet shows incomplete digestion, while the last tube without the violet color indicates the diastatic activity of the urine and from it "d" is calculated. The diastatic activity is expressed by $d \frac{38°}{30'}$, which represents the number of c.c. of 0.1 per cent. starch solution which 1 c.c. of the diluted urine can digest at 38 C. in thirty minutes to products not yielding a blue color with iodin.

The *urea* content of the twenty-four-hour specimens of urine was determined by the Marshall method.[6]

The principle of Marshall's method consists in the conversion of the urea into ammonium carbonate by means of an enzyme, urease, present in an extract of the soy bean, and the titration of the ammonia with standard hydrochloric acid and methyl orange, directly or after its removal with an air current.

The freezing point of serum was made in the ordinary way, utilizing the Beckman apparatus, 25 to 50 c.c. of blood usually being taken.

The blood urea was determined according to Marshall's[7] method.

The total non-protein nitrogen of the serum was determined as follows: Ten c.c. of serum was added to 115 c.c. of 95 per cent. alcohol, 100 c.c. of the filtrate being evaporated to dryness. The residue was subjected to Kjeldahl nitrogen determination. The result represents the nitrogen in 8 c.c. of blood.

The values which we accept as normal are as follows: Phthalein, 50 to 60 per cent. for one hour, 60 to 80 per cent. for two hours, $d \frac{38°}{30'} = 5$ or more, freezing point of serum —.56°, total non-protein N of blood .22 — .26 gm. per L. as determined by Folin, and blood urea .20 — .30 gm. per L. Retention of a mild grade occurs in many conditions without apparently serious renal involvement. Only when the retention is considerable do we consider the findings of importance. Such retention we refer to as cumulative phenomena, which means that the freezing-point of blood is at least —0.60 C., the total non-protein nitrogen 0.500 gm. and the urea of blood 0.550 gm. per L.

5. Brown and Smith: Bull. Johns Hopkins Hosp., July, 1914.
6. Marshall: Jour. Biol. Chem., 1913, xiv, 283; xv, 495.
7. Marshall: Jour. Biol. Chem., 1913, xv, 487.

TABLE 1.—GROUP A, MILD NEPHRITIS

No.	Name	Date	Clinical Diagnosis	Phthalein Per Cent.		Diastase 38° d — 30′	Albumin	Urea in 24-hr. Urine Gm.	Cryoscopy —( )°	Urea per L. Blood Gm.	Total Nonprotein N. per L. Blood Gm.	Remarks
				1 hr.	2 hr.							
1	B. 31744	3/26/13	Chronic nephritis; psychasthenia	37	21	0	+	13.5	0.56	0.380	....	Slight elevation of blood-pressure. Trace albumin and few casts in urine. Lactose excreted in 6 hours, 64 per cent.
2	P. Harriet Lane	2/12/13	Orthostatic albuminuria	5	55	2.5	—	8.3	....	....	....	Patient child of 12 years. Albumin disappeared in recumbent posture.
3	T. 30165	1/18/13	Febrile albuminuria; mild typhoid fever	42	20	1.7	+	....	....	....	0.530	Trace of albumin present after recovery.
4	S. 30162	1/ 3/13	Acute nephritis.....	30	23	5	+	...	0.58	...	0.160	Nephritis following turpentine ingestion. Recovery without albuminuria. Guaiac positive.
5	P. 30492	3/12/13	Chronic nephritis; arteriosclerosis	25	15	1.4	++	....	....	....	0.420	Lactose, 6 hours. Discharged, improved, March 14, 1913.
6	P. 30620	3/18/13	Chronic nephritis; neurasthenia	26	18	5	+	....	....	....	....	Discharged March 12, 1913.
7	H. 30506	3/ 7/13	Chronic nephritis; acute exacerbation	20	21	2	++	15.4	0.64	....	....	March 7, 1913, guaiac positive.
		3/31/13	.....	....	....	0	±	....	....	....	....	March 31, 1913, guaiac positive. Discharged April 19, 1913, improved.
8	C. 31673	11/ 8/13	Chronic nephritis; albuminuria	60	15	10	±	....	....	....	0.384	Discharged Nov. 14, 1913, unimproved.
9	C.	12/20/13	Chronic nephritis....	49	16	20	±	....	....	....	0.185	
10	L.	2/ 9/14	Albuminuria; mild nephritis	51	9	3.3	....	....	....	0.120	0.294	Lactose normal.
11	S. 32071	2/ 3/14	Acute nephritis.....	23	31	5	+	....	....	0.390	0.472	Lactose 12 hours. Guaiac positive. Discharged Feb. 18 1914.
12	C. 32178	3/ 8/14	Mild nephritis.....	47	15	6.6	+	....	....	0.210	0.455	Lactose 6 hours. Guaiac positive. Discharged March 14, 1914. improved.

RESULTS

In a series of 56 cases of medical nephropathies, 60 diastase, 41 phthalein, 30 urea determinations in the urine, together with 27 freezing points, 44 urea or total incoagulable nitrogen estimations in the blood-serum have been made. Opportunity to compare the functional findings with the anatomical changes present in the kidney at autopsy has been afforded in fifteen instances. For ease of presentation and discussion of the results, the cases presented in the accompanying table have been grouped as previously[8] into Section A, mild nephritis; B, severe nephritis; C, myocardial insufficiency; D, cardiorenal disease.

Group A—Mild Nephritis.—Twelve cases fall into this group. The lowest phthalein encountered was 40 per cent. for two hours, yet no diastatic activity could be detected in two cases, while $d \frac{38^{\circ}}{30'}$ was less than 2 in two other instances. Details concerning two of these cases can be seen from the following case reports:

CASE 1.—W. B., 31,744. The patient, a physician, aged 45, consulted Dr Barker concerning his shortness of breath. His family history was not of particular interest, nor was his past history, except for two attacks of gout and occipital headache persisting for years. Under strain the patient became weak, nervous and irritable.

For some years he had been aware of the fact that he had a slight albuminuria, a few casts and a slightly increased B. P. The blood picture was practically normal. The eye grounds normal except for slight blurring of upper and nasal margin. Except a B. P. varying from 130 to 160 the physical examination revealed nothing of importance. The Wassermann was negative.

CASE 5.—J. B. P., 30,492.—This patient, 64 years of age, had had the ordinary diseases of childhood, pneumonia as a young man, one attack of gonorrhea followed by stricture, pyorrhea alveolaris for twenty years and an attack of rheumatism fourteen years ago. For four years he has had to urinate once during the night.

Present illness dated back six weeks, starting with slight pain in region of left kidney. Urinalysis showed albumin and casts, whereupon his family sent him in for study.

Physical examination revealed slightly palpable radials, B. P. 135, an occasional extrasystole which later disappeared, and a faint aortic systolic murmur which frequently disappeared in erect posture. The Roentgen-ray showed slight dilatation of the arch. The blood picture was normal and the eye grounds showed slight blurring of part of margin of disc. The vessels were slightly tortuous, two small patches suggesting exudate in the left eye. There were no scars or hemorrhages in either eye.

In the remaining eight cases the diastatic findings were in harmony with those of the other tests, two cases showing but a slight reduction in diastatic activity, the others appearing normal.

The findings in Case 7 are interesting. This was considered an acute exacerbation, mild in type, in the course of a chronic nephritis. However, the phthalein was considerably reduced, 41 per cent. for

8. Rowntree and Fitz: THE ARCHIVES INT. MED., 1913, xi, 258.

TABLE 2.—Group B, Severe Nephritis

No.	Name	Date	Clinical Diagnosis	Phthalein Per Cent.		Diastase 38° d — 30′	Albumin	Urea in 24-hr. Urine Gm.	Cryoscopy —( )°	Urea per L. Blood Gm.	Total Nonprotein N. per L. Blood Gm.	Remarks
				1 hr.	2 hr.							
13	M. 30537	4/10/13	Chronic diffuse nephritis	±	±	1.4	+	....	0.58	....	....	Discharged April 29, 1913, improved.
14	K. M. ...	3/ 1/13	Chronic diffuse nephritis; arteriosclerosis	....	....	2.5	....	20.3	0.59	0.846	....	Diastase at March 12, 1913, 0. Patient better; discharged March 18, 1913.
		3/12/13	.....	...	....	0						
15	R. 30228	1/18/13	Chronic diffuse nephritis	±	±	1.7	+++	....	....	....	1.000	Discharged Feb. 20, 1913, improved.
16	R. 30606	3/25/13	Chronic diffuse nephritis; arteriosclerosis	10	14	1.3	±	10.0	0.53	0.460	....	Discharged March 31, 1914, improved.
17	R. 30585	3/25/13	Parenchymatous nephritis	....	...	0	++	10.5	....	....	....	Guaiac ++. Discharged March 24, 1913, unimproved.
18	N.	12/ 9/12	Chronic nephritis; hemiplegia	....	....	1.3	0	....	0.61	....	....	Died one month later.
19	[illegible] 30253	3/18/13	Parenchymatous nephritis	58	12	5	++	11.5	0.50	....	0.160	Persistent edema over six months, heavy albuminuria; retention of NaCl.
20	[illegible] 30073	12/12/12	Chronic diffuse nephritis; uremia	±	±	1.7	++	....	....	....	0.880	Died. Incoag. N 2.00 just before death.
21	S. 29980	12/ 8/12	Chronic diffuse nephritis; gout	±	±	1.7	+	....	....	....	2.000 0.590	Necropsy 3847. Discharged Dec. 14, 1912, improved.
22	McC. 30286	1/21/13	Chronic diffuse nephritis; arteriosclerosis	30	14	1.7	±	....	....	....	0.080	Discharged Jan. 22, 1913.
23	W. 31769	10/ 8/13	Chronic nephritis....	10	7	5	....	...	....	....	1.030	Discharged Dec. 12, 1913, improved.
24	R. 31674	11/26/13	Chronic nephritis....	8	10	10	++	....	....	0.414	0.588	
25	F. 31862	12/19/13	Chronic nephritis....	22	18	2	+	....	....	....	0.322	Discharged Jan. 7, 1914, improved.
26	W.	1/28/14	Chronic nephritis....	30	6	0	+	....	....	0.360	0.420	Lactose 12 hours.

two hours (20 per cent. for first hour), which is the lowest phthalein in this group. $D\frac{38°}{30'}$ was only 2, while the freezing point was reduced to —.64. All the findings are in harmony and point to a more serious involvement than clinically was thought to exist. The drop in $d\frac{38°}{30'}$ to 0 three weeks later, at which time the albumin had practically disappeared, is inexplicable as the patient seemed much improved in every way and was discharged in good condition.

No evidence of cumulative phenomena has been encountered in this group except in the instance just referred to, in which the finding was in keeping with the phthalein and diastase findings.

It appears, therefore, that findings of all the tests are in harmony in nine of the twelve cases and that the findings of all the tests, with the exception of diastase, are in accord throughout. The diastatic activity has indicated severe functional involvement in three cases of nephritis in which the clinical picture, history, phthalein test, tests of retention and the subsequent course of events, all showed that the involvement was but slight.

Group B—Severe Nephritis.—Fourteen cases of severe nephritis were studied. The diastatic activity was decreased in all but three instances — $d\frac{38°}{30'}$ being 1.7 or lower in ten cases. Two of these patients died, but only one came to necropsy. Zero value for diastase was encountered three times. K. M., No. 14, showed a zero value shortly before leaving the hospital, at which time clinically he seemed much improved. Normal diastatic values were found in Nos. 19, 23 and 24.

In No. 19 the finding is in harmony with all other tests with the exception of salt, toward which a retention existed. The case is of great interest as an instance of hyperpermeability in nephritis and has been reported in detail. Normal diastase findings in Nos. 23 and 24 are irreconcilable with other findings, since the phthalein was markedly decreased and cumulative phenomena were present in both cases.

Depression of the freezing point to —.61 was only once encountered (No. 18), the patient dying one month later. In the presence of very severe nephritis, the freezing point was not markedly decreased in three instances (Nos. 13, 14 and 16). Cumulative phenomena as evidenced by urea and total non-protein N of the blood were present in six cases. The low N content in Case 19[9] (referred to above) is of interest in connection with the high freezing point and normal phthalein

9. This case has been classed as severe nephritis because of clinical findings—marked albuminuria and considerable edema. The findings of the functional studies are prognostically correct, since the patient's condition is now no worse than it was when she was observed two years ago.

TABLE 3.—Group C, Cardiac Cases

No.	Name	Date	Clinical Diagnosis	Phthalein Per Cent. 1 hr.	Phthalein Per Cent. 2 hr.	Diastase 38° d — 30′	Albumin	Urea in 24-hr. Urine Gm.	Cryoscopy —( )°	Urea per L. Blood Gm.	Total Non-protein N. per L. Blood Gm.	Remarks
27	S. 30622	4/ 9/13	Myocardial insufficiency; aortic and mitral insufficiency; mitral stenosis	....	....	10	+	5.1	....	....	....	Died three days after tests.
28	B. 30675	5/29/13	Myocarditis; miliary Tb.; pericarditis; chronic passive congestion	18	10	0	0	....	....	....	....	Extreme chronic passive congestion at autopsy. Kidneys otherwise normal.
29	K. 30955	6/ 5/13	Arteriosclerosis; myocarditis	....	40	2.5	+	....	....	....	....	Broken compensation present at time of test. Lactose 9 hours. Dicharged Aug. 8, 1913, improved.
30	J. 30704	5/29/13	Myocardial insufficiency; aortic insufficiency; myocarditis	23	18	0	+++	....	....	....	....	Died in broken compensation, which was severe at time of all tests. Kidneys showed only chronic passive congestion.
		6/ 5/13	.....	....	....	5	+++					
31	B. 30625	4/ 9/13	Myocardial insufficiency; aortic insufficiency; arteriosclerosis	....	....	10	0	....	....	....	....	Convalescing at time of test. Discharged April 13, 1913; condition improved.
32	L. 30618	3/31/13	Myocardial insufficiennncy...	....	.. .	1.4	+	3.7	0.54	....	....	Moderate broken compensation at time of test. Discharged June 24, 1913, unimproved.
33	H. 30380	3/ 7/13	.....	....	.. .	3.3	+	8.1	....	....	....	Patient died without recovering compensation. Autopsy 3915.
		3/26/13	Acute endocarditis; acute lobar pneumonia; polyserositis	....	....	10	+	19.3	0.56	0.355	....	
34	J. 30461	3/ 7/13	Myocardial insufficiency; aortic and mitral insufficiency	....	....	10	0	17.6	....	....	....	Broken compensation at time of test. Discharged April 3, 1913, improved.
35	E. 30396	2/25/13	Myocardial insufficiency; aortic and mitral insufficiency	29	25	3.3	++	10.2	0.48	0.168	....	Severe broken compensation; death without recovery. Kidneys normal at autopsy except chronic passive congestion.
36	C. 30178	1/18/13	Myocardial insufficiency; pericarditis; myocarditis	....	....	2.5	+	....	0.56	....	....	Died. Kidneys normal at autopsy, 3870.
37	A. 30201	1/17/13	Myocarditis	36	19	1.7	+	....	0.67	....	0.470	Autopsy: Chronic passive congestion, myocarditis. Kidneys otherwise normal. 3858.
38	K. 30602	5/ 2/13	Myocardial insufficiency; mitral insufficiency; arteriosclerosis	16	17	0	+	13.4	....	....	....	Died May 3, 1913.
39	M. 30503	3/ 7/13	Myocardial insufficiency; aortic insufficiency; arteriosclerosis	.. .	....	0	±	8.6	0.58	0.432	....	Guaiac +. Discharged March 28, 1913, improved.
40	H. 30666	3/10/13	Myocardial insufficiency; arteriosclerosis	....	....	2.5	—	13.4	....	....	....	In broken compensation at time of test. Discharged May 13, 1913, improved.
41	G. 30499	3/ 7/13	Mitral stenosis; mitral insufficiency; hemiplegia	....	....	3.3	±	39.7	....	....	....	Severe broken compensation at time of test. Died. Kidneys normal except for chronic passive congestion.

and normal diastatic activity. The rapid increase in the total non-protein N in Case 20, reaching 2 gm. per L. just prior to death, is also worthy of note.

No serious disagreement in the findings of the various tests in this group is encountered, except that diastatic activity is normal in two cases of severe nephritis which show cumulative phenomena.

In considering diastase as an index of the functional capacity of the kidney in uncomplicated nephritis, it is evident that in the majority of instances it is of some value. In three cases of mild nephritis, however, severe involvement of function has been indicated where the other tests and the clinical history fail to substantiate this, while in two cases of severe nephritis with very low phthalein output and with cumulative phenomena, no decrease at all in functional capacity is indicated. Since its findings are entirely out of harmony in five cases in a series of twenty-six, obviously no absolute reliance can be placed in it. The test must be considered of only corroborative[10] value.

Group C — Cardiac Cases. — Fifteen cardiac cases, myocardial insufficiency, endocarditis, etc., unassociated with nephritis were studied, eight of them coming to necropsy. As previously pointed out, these cases rarely have the markedly decreased phthalein output which is seen in severe grades of nephritis, although they may present a similar clinical picture. Only when the passive congestion is of an extreme grade is the phthalein output much reduced. With restoration of compensation, the phthalein rapidly becomes normal again. Of the six cases in which the phthalein output was studied, five patients died. Two had more than a 50 per cent. output, two 40 per cent., one 33 and one 28 per cent. In severe myocardial breaks there is some tendency to a reduction of the output for the first hour (the highest output for the first hour was 36 per cent.), with a fair output the second hour. In many instances the outputs for the first and second hours are nearly equal. In this condition the first hour excretion is often a truer index to function than that for two hours.

Diastase determinations were made in all these cases. Three patients died with a perfectly normal diastatic activity, although two of these had earlier showed a decreased content. Four others died with a fair activity, two with a zero output, while two did not die, although the diastase was very low — zero in one instance. Therefore, little dependence, prognostically or diagnostically, can be placed on the diastase findings.

As has been previously pointed out by Strauss and Hohlweg, marked increase in blood urea and total non-protein N is not fre-

10. Where the findings of the diastase test conflict with those of the phthalein, blood urea or blood nitrogen, we would discard the former.

TABLE 4.—Group D, Cardio-Renal Cases

No.	Name	Date	Clinical Diagnosis	Phthalein Per Cent. 1 hr.	Phthalein Per Cent. 2 hr.	Diastase 38° d — 30′	Albumin	Urea in 24-hr. Urine Gm.	Cryoscopy —( )°	Urea per L. Blood Gm.	Total Nonprotein N. per L. Blood Gm.	Remarks
42	Z. 31821	12/12/13	Chronic nephritis; myocardial insufficiency	60	13	2.5	++	....	....	....	0.330	Discharged Dec. 13, 1913, improved.
43	J. 30469	2/25/13	.....	....	....	3.3	+	15.9	0.57	0.480	....	Mild broken compensation at time of test. Discharged March 14, 1913, improved.
		3/ 7/13	Myocardial insufficiency; aortic and mitral insufficiency; chronic nephritis	21	5	5	+					
44	B. 30117	12/21/12	Myocardial insufficiency; chronic nephritis	14	21	....	++	....	0.58	....	0.320	In broken compensation at time of test. Discharged Feb. 19, 1913, improved.
45	G. 31757	1/ 3/13	Myocardial insufficiency; chronic nephritis	51	15	20	+	....	0.56	....	0.340	Convalescent at time of test.
46	B. 30599	3/25/13	Myocardial insufficiency; chronic nephritis	30	13	2.5	++	20.1	.. .	....	0.050	In broken compensation at time of test. Discharged April 9, 1913, improved.
47	J. 30482	3/12/13	Myocardial insufficiency; hemiplegia; chronic nephritis	40	18	5	+	30.2	.. .	....	0.360	In broken compensation at time of test. Died.
48	S.	5/29/13	Myocardial insufficiency; mitral insufficiency; arteriosclerosis; chronic nephritis	12	45	0	....	....	....	....	....	Fair compensation at time of test.
49	B. 30486	3/ 7/13	Myocardial insufficiency; chronic nephritis	44	11	5	+	19.8	0.59	0.280	0.280	Discharged Mar. 21, 1913, improved.
50	S. 30652	4/ 9/13	Myocardial insufficiency; aortic insufficiency; arteriosclerosis; chronic nephritis	40	17	1.3	—	19.8	0.60	0.460	....	In moderate broken compensation at time of test. Discharged May 5, 1913, improved.
51	C. 30679	4/ 9/13	Myocardial insufficiency; aortic insufficiency; chronic nephritis	....	....	0	+	6.3	....	....	....	In moderate broken compensation at time of test. Discharged April 11,1913, improved.
52	G. 30554	4/ 2/13	Myocardial insufficiency; myocarditis; acute endocarditis; chronic diffuse nephritis	. ..	....	0	+	19.4	0.50	1.100	....	Chronic passive congestion of kidneys; otherwise normal at autopsy. 3900.
53	G. 30658	4/ 2/13	Myocardial insufficiency; arteriosclerosis; chronic nephritis	....	....	0	+	19.1	0.55	0.650	....	In broken compensation at time of test. Discharged May 28, 1913, improved.
54	J. 30580	3/26/13	Myocardial insufficiency.....	....	....	5	++	....	....	....	....	Discharged Mar. 30, 1913, improved.
55	J. 31796	12/18/13	Myocardial insufficiency; chronic nephritis	...	60	10	±	....	....	0.300	0.322	Discharged Dec. 30, 1913, improved.
56	R. 31752	11/27/13	Myocardial insufficiency; chronic nephritis	48	20	2.5	+	....	. ..	....	0.518	Discharged Dec. 10, 1913, improved.

quently seen[11] in pure passive congestion. A cumulative phenomenon is only seen once in six cases in which such studies were made, e. g., Case 37 in which the freezing point was —.67 and total incoagulable nitrogen on the upper limit of normal, .47 gm. per L.

Group D—Cardiorenal Cases.—Fifteen cases fell into this group, three of which ended fatally. The relative and absolute degree of cardiac and renal involvement varied much in the different cases, so that practically all types of cardiorenal cases are included.

One patient (No. 47) died showing a normal diastase during a cardiac break in nephritis. The phthalein here was 40 per cent. first hour and 18 per cent. second hour. Two patients (Nos. 52 and 53) died with a zero output and one of these showed only a chronic passive congestion at necropsy. Two (Nos. 48 and 51) left the hospital improved after exhibiting a zero output. In six cases a normal diastase was encountered, but in three of these the renal function was good as indicated by the other tests.

It has been previously pointed out that diseases of the kidney may be clinically identical, but functionally and pathologically different, and that by the aid of the phthalein test it is possible to determine in any given cardiorenal case whether the heart or the kidney is relatively more responsible for the clinical picture presented. This is not possible by the diastase test, since the diastatic activity seems to be markedly but inconstantly depressed in both cardiac and renal disease.

Cumulative phenomena were encountered in only three cases, all of which died. Case 52 is of special interest as the necropsy showed no changes other than passive congestion, although the blood urea was 1.1 gm. per L. The freezing point was at the same time not at all depressed.

Influence of Blood and of Albumin on Diastatic Activity.[12]—In the foregoing table blood is recorded in the urine in seven instances. In the other cases microscopical examination or the guaiac test failed to show its presence. Three cases of mild nephritis showed blood and a normal or high "d." A mild nephritic on two occasions showed a low "d," one severe nephritic and one cardiac case a zero "d" value in the

11. We have now seen three cases in which cumulative phenomena have been encountered in pure chronic congestion, e. g., Cases 37 and 52 and a third case which has been reported by Marshall and Davis.

12. As stated above, this test deals only with "diastatic activity" and not with "diastase content." The activity is dependent on environment and not on actual amount of diastase. Only the influence of albumin and blood have been considered here, since they have entered into the interpretation of the findings of the test in the hands of certain authors.

presence of blood. According to Wohlgemuth[13] and to Corbett,[14] blood accelerates diastatic activity.

Wohlgemuth[15] and Neumann[16] do not consider that the presence of albumin affects the diastatic activity of the urine, while Corbett and Geyelin[17] think that it may, claiming that higher findings are encountered in cases with marked albuminuria.

In the sixty diastase determinations made, the presence or absence of albumin was noted in fifty-six instances, with forty-eight positive findings. Exact quantitative studies of the albumin content were not made, the amount present being indicated as a trace, +, + +, etc.

In the twelve cases of mild nephritis six cases showed a normal or high "d" value. In none of these was there much albumin in the urine, but in three the guaiac test was positive. Only once (Case 7) was much albumin present, the "d" value was low, but later with less albumin the "d" sank to 0 — blood still persisting in the urine.

In fourteen cases of more severe nephritis much albumin was present in five instances and in two of these a normal "d" was encountered. One of these cases (No. 19) had normal function indicated by all other tests except the salt test, so that it is not necessary to assume that the albumin was responsible for the high "d" value in this instance. In Case 17, despite the presence of large amounts of albumin and the presence of blood, a zero "d" value was present.

In fifteen cardiac cases much albumin was present in three instances and a normal diastase value only once. Case 29 with about the same amounts on each occasion gave a 0 value at first and a normal one later. The patient died. The phthalein here showed definite functional impairment, 23 and 18 per cent. for the first and second hours, respectively—the first hour reading being probably the truer index to the function.

In fifteen cardiorenal cases much albumin was present in four cases and normal diastase once in three of the four cases in which it was determined. In the series of severe renal and cardiorenal cases, twelve instances of marked albuminuria were found and in only four cases was "d" normal or higher, while in four it was 1.7 or 0.

Analysis of our data does not indicate that albumin plays a great rôle in activating diastase. Are we justified, then, in ascribing high or normal values in the presence of marked albuminuria to activation of diastase by the albumin? In Case 19 we have an instance of hyper-

13. Wohlgemuth: Ztschr. f. Urol., 1911, v, 801.
14. Corbett: Quart. Jour. Med., 1913, vi, 365.
15. Wohlgemuth states that with the technic here employed no activation occurs unless the albumin is present in large amount.
16. Neumann: Deutsch. Arch. f. klin. Med., 1913, cxi, 164.
17. Geyelin: THE ARCHIVES INT. MED., 1914, xiii, 96.

permeability of the kidney—high phthalein, 70 per cent. for two hours, normal diastase, normal urea output, no cumulative phenomena but retention of salt. It is not necessary to assume that the diastatic activity has been accelerated by the albumin.

First it seems advisable to determine whether or not albumin does activate diastase, and if it does, to what extent. In case it does, then it must be determined whether the character of the albumin or the quantity plays a rôle and to what extent. Since albuminuria is almost constant in conditions in which the diastatic activity is determined, and since marked albuminuria may be associated with zero and low "d" values, it constitutes a serious defect in the test to attempt to ascribe normal or high findings in certain instances to the presence of albumin. Uncertainty as to the interpretation of the findings of a test must of necessity decrease the practical value of that test.

CONCLUSIONS

1. The quantitative estimation of the diastatic activity of the urine as it is employed at present shows low values in the majority of cases of mild and severe nephritis, while in cardiac and cardiorenal cases the diastase findings are bizarre. Owing to the frequent occurrence of normal diastatic values in cases in which considerable or grave renal functional involvement is unquestionably present, and of low diastatic values which are not in accord with the clinical course of the case or with findings of other functional tests, no diagnostic or prognostic significance attaches to this test, other than that which is corroborative in character. As a single test it is unreliable. Further data as to the influence of albumin on the "d" value are desirable.

2. The phthalein test is the one of choice and unquestionably the most valuable single test in this group of cases.

The total non-protein N and urea content of the blood are of about equal value in severe cases, while the freezing point of the serum is probably of somewhat less value since depression in the freezing point is lacking in several instances in which one or both of the other tests indicate that retention is present.

3. Both tests of excretion and of retention are valuable. In all cases a phthalein test is advisable. *Wherever the phthalein output is decreased even but slightly the total non-protein nitrogen or the blood urea or both should be determined.*

THE EFFECT OF SODIUM SALICYLATE ON VARIOUS TYPES OF EXPERIMENTAL ARTHRITIS*

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The following experiments were made in order to determine the effect of salicylates in animals inoculated with various types of streptococci. These cocci were isolated from various sources, and their causal relation to certain clinical conditions, especially of the rheumatic types, is pointed out in its appropriate place with the several series of experiments reported below.

The experiments with the different organisms were all carried on in the same way. The animals used were rabbits. They were given, in some cases on the previous day, in other cases from one to two hours previous to inoculation, 5 grains of a synthetic preparation[1] of sodium salicylate subcutaneously or intramuscularly. They were then inoculated intravenously with suitable doses of living suspensions of twenty-four-hour cultures of the streptococci. On each subsequent day from 3 to 5 grains of salicylate were given to the animals, usually subcutaneously, for a period of two to three weeks if the animal lived, or until the animal died. Careful observations of the animals were made from day to day for evidence of infection, especially arthritis. In all experiments a series of six animals were inoculated with identical amounts of culture, three of which received the salicylate as above indicated, and three served as control animals. Young animals were used, since they are more susceptible to infection than old animals.

Experiment 1.—Six young rabbits were carefully selected, of approximately the same weight and age and about three-quarters grown. Their weight was about 1,100 gm. All were given, into the ear vein, equal amounts of Streptococcus 236 suspended in about 2 c.c. of salt solution. Three animals had received two hours previously 5 grains of sodium salicylate subcutaneously; this same amount was continued daily following the injection. Streptococcus 236 is an organism that was isolated from a person dying of an infection which began as a sore throat and which occurred during the Chicago milk epidemic in 1912. It was hemolytic and encapsulated and possessed the properties of the epidemic type of streptococci as described by Dr. Rosenow and myself previously.[2] The reasons for testing an organism of this type are readily appar-

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1. It has been shown by Waddell (ARCHIVES INT. MED., 1911, viii, 784) that the synthetic preparations of salicylates behave like the natural products.

2. Davis, D. J., and Rosenow, E. C.: An Epidemic of Sore Throat Due to a Peculiar Streptococcus, Jour. Am. Med. Assn., 1912, lviii, 773.

ent, since the use of salicylates, aspirin, etc., is so universal in cases of severe sore throat, especially when complicated by joint symptoms as not infrequently happens.

In this series all the animals died, each having developed multiple arthritis. The three receiving salicylates lived four, seven and three days, a total of fourteen days following inoculation. The controls lived eight, eleven and seven days, a total of twenty-six days. Two of the former and one of the latter showed slight lesions of endocarditis. From the joints in all the animals the streptococcus was recovered and in four of the six the heart's blood at necropsy yielded streptococci. It is evident from this experiment that the salicylates had no favorable influence on the course of the infection with this streptococcus. Indeed, the data indicate rather a slight unfavorable effect.

Experiment 2.—The next series of animals were inoculated with a hemolytic streptococcus of bovine origin isolated from the udder of a cow suffering with severe mastitis. It was very similar to Streptococcus 236 and was highly virulent to animals. The experiment was made exactly as was the previous one. All the animals developed multiple arthritis. The three receiving salicylates died after five, two and three days, respectively. Two of the controls died after five and three days and the remaining animal was still alive one month later though suffering from a large and badly deformed joint. One of the salicylate rabbits at necropsy revealed endocarditis. In the heart's blood of the five animals that died streptococci were found post mortem. Here again the salicylates had no favorable effect; if anything the reverse.

Experiment 3.—The next series of animals was treated exactly as in the previous experiments except that after the first dose of 5 grains, 3 grains of salicylates were given daily. This of course is still relatively a large dose. The streptococcus used was obtained from Dr. E. C. Rosenow, who shortly before had isolated it from the kneejoint of a person suffering with acute rheumatic fever. This organism had the general properties of the *Diplococcus rheumaticus* as described by Poynton and Payne. Since these organisms are less virulent for animals than the hemolytic streptococci, it was necessary to inject considerably larger doses, the growth from three blood-agar slants being given to each animal. All the animals without exception developed arthritis of greater or less intensity and multiple in character. Only one, an animal which was receiving salicylates, died; this occurred nine days after inoculation and the necropsy revealed multiple acute arthritis, endocarditis and fibrinous pericarditis. Cultures were positive from all these lesions. Three of the animals were killed after eleven days and cultures from joints yielded streptococci. The remaining two animals, one receiving salicylates daily for three weeks, and the other as control, were kept under observation for three months. No appreciable effect of the salicylates was evident. At the end of this time the joints in both animals were still distinctly and about equally enlarged. Otherwise the animals appeared normal and were not emaciated.

Experiment 4.—The next series of animals was inoculated with a streptococcus (256) isolated from the tonsil crypts in pure culture from an individual suffering with chronic tonsillitis and chronic rheumatoid arthritis. This organism was probably the causal agent in this condition. It was hemolytic and highly virulent for animals. The experiments were carried on exactly as with the other organisms. All animals developed multiple joint lesions within a few days. Those receiving the salicylates died after five, eight and nine days, respectively. No endocarditis. Two of the control animals died after four and five days; the third animal did not die, but developed multiple arthritis, the lesions becoming chronic and leading to marked enlargement and deformity of some of the joints. In connection with this experiment another rabbit was inoculated with a small dose of the same streptococcus (256). Joint lesions appeared after several days. Seven days after the inoculation and at a time when the arthritis was distinct, the animal was given 5 grains daily of sodium

salicylate. The lesions continued in a chronic state and showed no appreciable improvement after several weeks. The general course of the infection in this animal was almost identical with the outcome in the control animal that lived in the previous experiment, though it had a somewhat smaller dose of streptococci. It must be said, therefore, that the sodium salicylate had no appreciable effect on the course of the infection.

SUMMARY

It is clear from the several series of experiments here reported that sodium salicylate does not exert a favorable effect on infections in rabbits caused by various types of streptococci under the conditions detailed above. It does not prevent localization of the organism in joints, nor does it prevent the appearance of endocarditis. It would seem to have, therefore, no prophylactic value, nor does it alter the course of the infection after it has once become established.

SKIAGRAPHIC STUDY OF THORAX, THORACIC WALL AND THORACIC VISCERA*

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The Roentgen-ray picture of the normal or abnormal thorax invariably shows certain opacities corresponding to the hilus of the lung. Latent tuberculosis is found in this same location, and it has been stated that tuberculosis always begins here and spreads through the lung in a peripheral direction. It is of prime importance, therefore, to ascertain if possible the cause of these opacities.

An attempt was made to analyze the roentgenogram of the thorax by taking a cadaver and making a Roentgen-ray plate of the thorax intact; of the thorax with heart and lungs removed; of the posterior thoracic wall alone; of the detached heart and lungs; of the heart separate; of the lungs freed from the heart and vessels, and finally of the lungs with the bronchi, artery, or vein injected with opaque emulsion of barium sulphate, or with citrated blood.

The cadaver was that of a negress, aged about 65, who died of gangrene of the legs due to extreme anasarca, probably of renal origin. No physical or other examination of the lung was made prior to death.

The roentgenogram of the intact thorax showed an enormous heart shadow. This roentgenogram was made with the subject lying on the back, the plate underneath and the tube 26 inches above the plate.

The roentgenogram of the intact thorax does not show quite so many opacities as usual beside the heart, because the heart shadow overlaps and obscures them. Nevertheless, a few opacities appear in the region of the hilus of the lung. A careful dissection later showed complete absence of calcified lymph-nodes; the numerous peribronchial lymph-nodes were pea-sized and of a dark, almost black, color. In Figure 2 the costosternal cartilages are seen, just to the right of the sternum. It is impossible to trace these cartilages throughout their extent, and hence they may at times cause shadows that might be mistaken for shadows of something else, as for instance, calcified nodes.

The lower lobe of the right lung shows an infiltrated condition, which was evidenced in the gross specimen by a sharply demarcated zone of deep red in contrast with the pale color of the normal lung

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* From the Pathological Laboratory, Austin Presbyterian Sanitarium.

above. This infiltration shows beautifully in Figure 6. It was easily detected by palpation of the specimen, as the lung was less crepitant in the affected region. The roentgenogram of the intact thorax gives only a hint of this condition. The failure to show this might be attributed to the fact that the tube was not centered directly over the region, and might suggest that an intensive study of the lung could be better made by a series of small roentgenograms made with the tube in each instance accurately centered over the area in question.

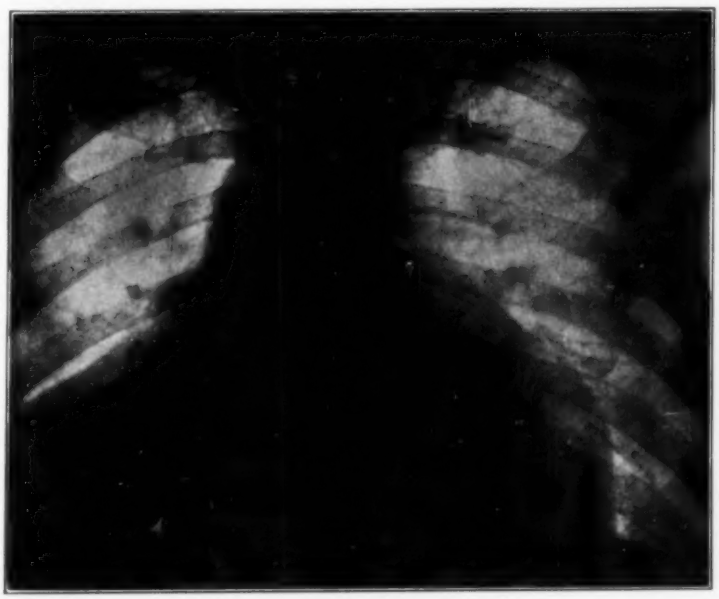

Fig. 1.—Intact thorax; picture made post mortem. Note the mottling seen in some of the intercostal spaces, especially on right side, low down. Heart is large, apex projecting beyond edge of plate.

The picture of this thorax does not show the bronchovascular tree, probably because the heart and pericardium overshadows the portion where the tree should show plainest, and because of the post mortem collapse of the vessels.

A comparison of Figure 2 with Figure 1 shows that practically all the opacities are due to the contents of the thorax and not to the thoracic wall. The single exception is the shadow due to the costo-sternal cartilages referred to above, and which is visible in Figure 2. The blood-vessels and lymph-nodes of the chest wall do not show at

Fig. 2.—Front and back thoracic wall with lungs and heart removed. Note absence of densities or obscurities in interspaces. Intercostal cartilages show very faintly near arrows.

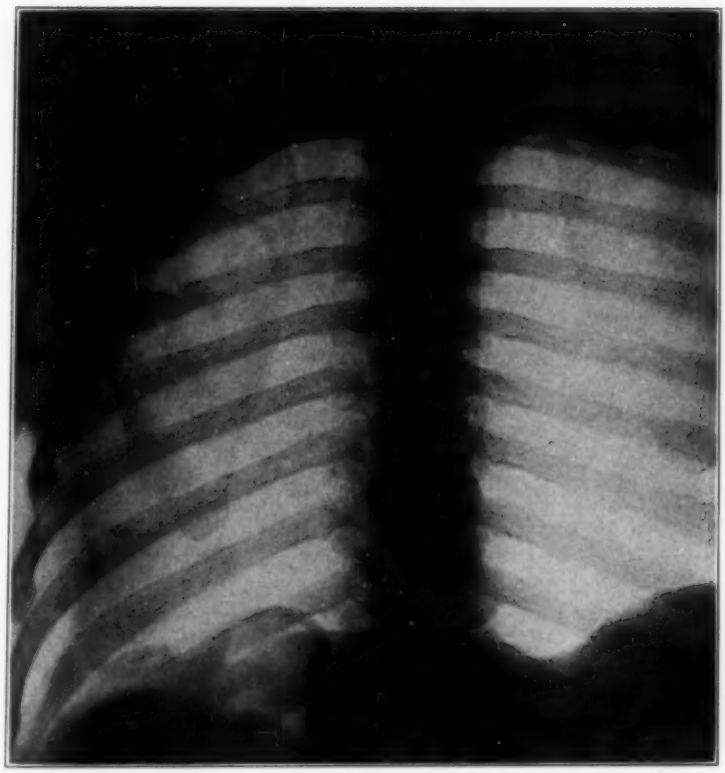

Fig. 3.—Posterior thoracic wall alone. Intercostal cartilages absent.

Fig. 4.—Heart and lungs after removal. Note consolidation of lower lobe of right lung. Hilus shadow shows on left side. Note the arborization of lung by bronchi and vessels.

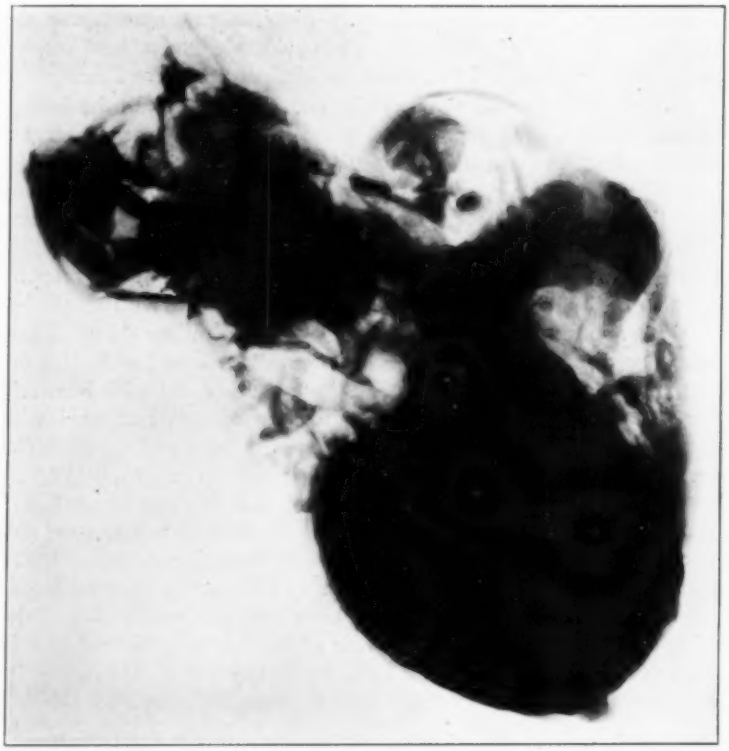

Fig. 5.—Heart and part of great vessels. Note that the heart, connective tissue, and vessels all obstruct the Roentgen rays.

all. Figure 3 is the equivalent of Figure 2, except that the anterior thoracic wall has been removed.

Figure 4 is instructive in that it shows many dense areas in the region of the hilus. These shadows are not due to lymph-nodes, because dissection proved that none of the nodes were calcified. It is difficult to account for all these densities on the ground that they are shadows of bronchi, because the bronchi (as will be noted later in this article) show as pairs of parallel lines with a rarefied area between. Certain it is that the bronchi do not satisfactorily account for all the hilus area. A part of the hilus density is evidently due to blood vessels, pericardium, mediastinal fat, and adventitious connective tissue.

Figure 5 presents all the densities and opacities due to the fibrous connective tissue in and around the arteries, veins and heart. Figure 5 stands in marked contrast to Figure 6, because the latter showing the lungs alone is comparatively free from densities and opacities. It seems that the contrast between the roentgenogram of the lungs alone (Fig. 6) and that of the heart and vessels (Fig. 5) brings out a valuable lesson, namely, that hilus shadows are due not only to the bronchi, but also to the blood vessels, and adventitious connective tissue.

Our studies were continued with lungs from healthy sheep. These organs are very similar to human lungs but differ in one rather important particular; namely, they have thin-walled bronchi with relatively large lumen. The density of bronchial shadows as compared with that of blood vessel shadows is brought out in Figures 7 and 8. The first of these shows sheep lungs and heart intact. The second (Fig. 8) shows sheep lungs with the heart removed while the blood-vessels and other little tags of mediastinal tissue remain. Note how much the picture is cleared up when the lungs are freed from the vessels (as far as possible). Figure 9 represents the lungs of the sheep after all blood-vessels and tags of mediastinal tissue are removed from the hilus. The minute density near the lower border of the left lung (seen better in Figures 8 and 10) is a calcified area, verified by actual dissection. Its occurrence in this sheep, selected at random, indicates that calcified areas are not uncommon, especially since we have found them in about 5 per cent. of human chests examined. We do not consider a dense area as necessarily a calcified area unless it is clearly defined and isolated.

The appearance of the heart and lungs in Figures 4 and 7 is in one respect unnatural, namely, in so far as heart and vessels are practically empty. It can be observed in these two pictures that the blood-vessels do not cast a distinct shadow. The blood content is lacking. Figure 11 and Figure 12 show the difference between the shadow cast by the pulmonary artery when empty and when distended

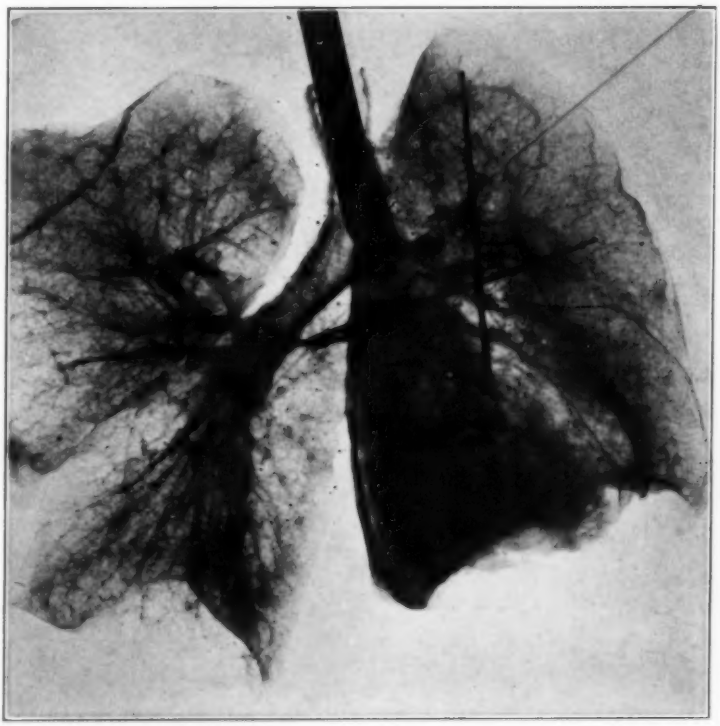

Fig. 6.—Lungs freed from heart and vessels. The probes are in arteries and veins, but these are collapsed and do not show. Note above all that each bronchus shows as two parallel dark lines with a light band between. Note the consolidated lower right lobe.

Fig. 7.—Heart and lungs of sheep.

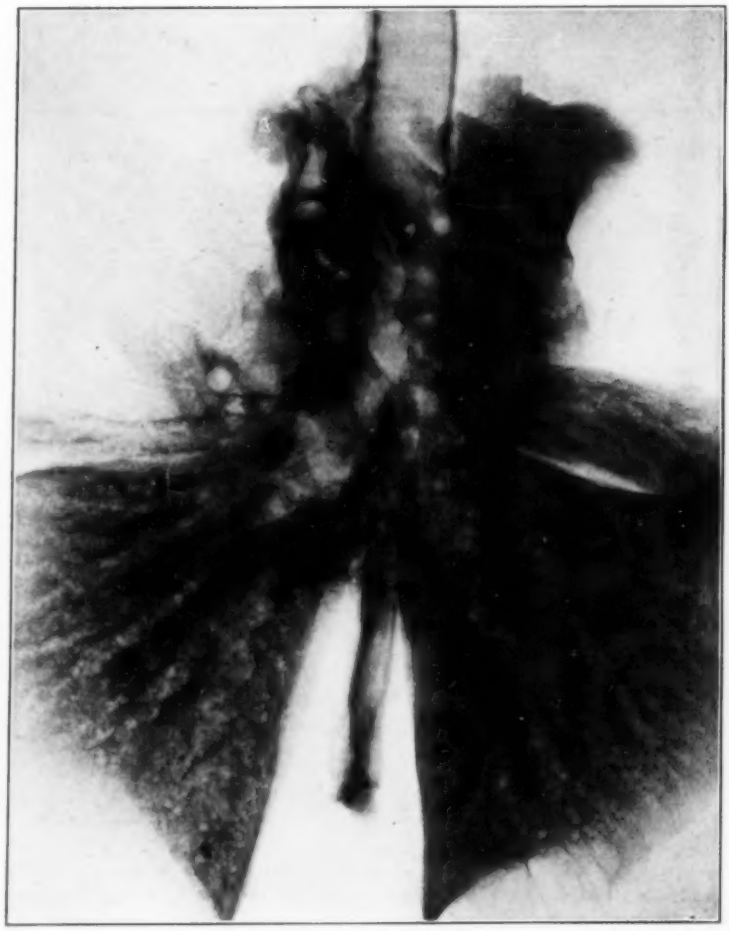

Fig. 8.—Same as Figure 7, except heart has been cut away, leaving great vessels, of which the mouths show as rings.

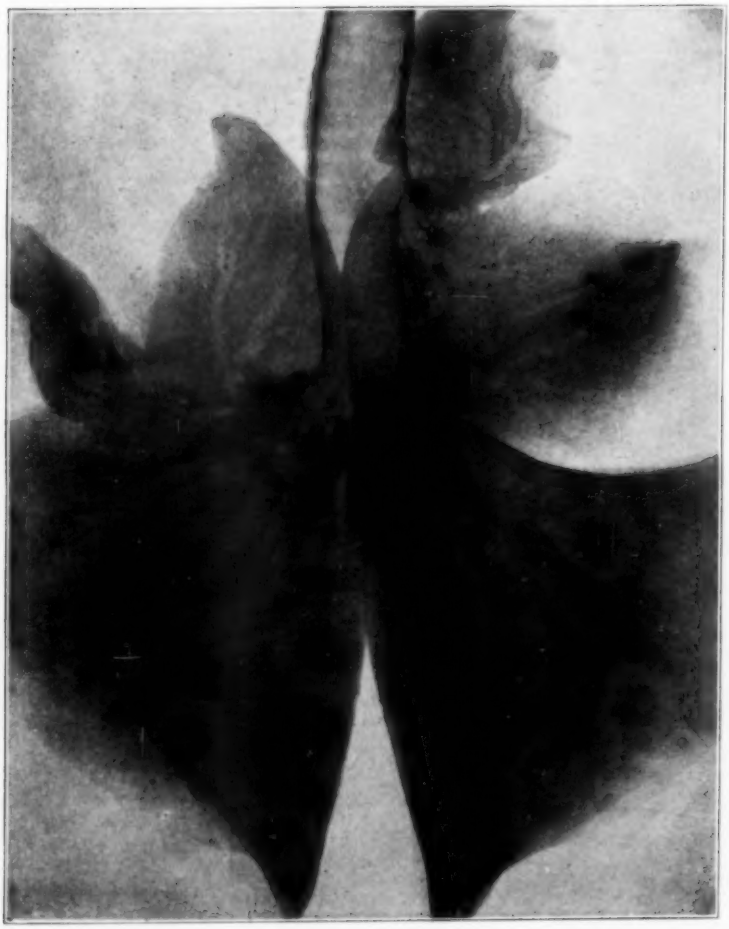

Fig. 9.—This is same as Figure 8, except that vessels are, as far as possible, cut away. Note absence of obscure hilus densities.

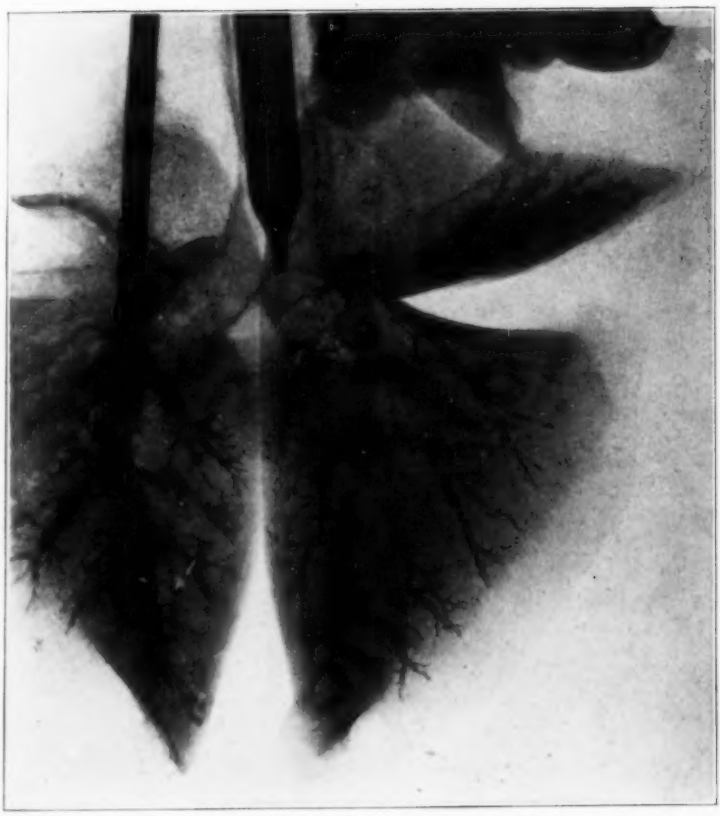

Fig. 10.—Same as Figure 9, but now injected with barium emulsion. The small lobe, bronchus alone injected. Large lobe on same side, artery alone injected. Large lobe on opposite side, vein alone injected. Note that the vessels accompany the bronchi and are of about the same size.

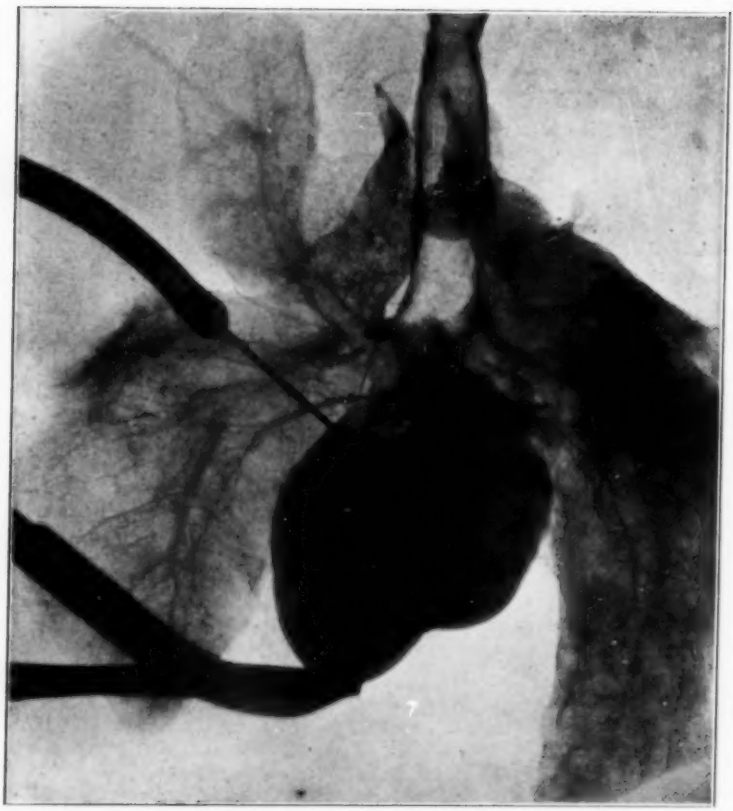

Fig. 11.—Sheep heart and lungs with vessels empty and collapsed; needles inserted ready to inject citrated blood.

by blood. The artery with its column of blood casts a distinct shadow in Figure 12. The relative position of all structures is identical in the two pictures, because the needle for injecting the blood was *in situ* before Figure 11 was made. Immediately after exposing the plate for Figure 11 the clamps were released and citrated blood was allowed to

Fig. 12.—Same as Figure 11, except clamps were released and citrated blood was allowed to run in without moving specimen, which is in almost exactly the same position as shown in Figure 11. Note the pulmonary artery shadow accompanying the primary bronchi. The arrow points to a branch of the pulmonary artery going to small upper lobe.

run into the pulmonary artery under six feet gravity pressure. Note that the artery with its contained blood casts a dense shadow, whereas the accompanying bronchi show negatively as vacuities except that the side walls of the bronchi seen in section show as a density.

Figure 13 shows the difference in the roentgenogram of a bronchus and that of an artery of about the same size. The artery is full of blood. It will be observed that the bronchus shows as a light streak (lumen) bordered by a dark streak (wall) on each side. The blood-vessel, however, shows as a uniformly dark band, except for the fact that in this instance an air bubble shows as a round light area.

None of the plates have been retouched, and the half-tones do not give an absolutely perfect idea of all the points.

CONCLUSIONS

1. The anterior and posterior chest walls offer no source of error in interpreting hilus shadows, except that the costal cartilages may be

Fig. 13.—Artery and bronchus. Note difference in character of shadow cast. The artery is distended by citrated blood. Note air bubble in artery.

irregularly calcified; this condition is easily recognizable in the roentgenogram.

2. When the lung and heart are removed from the thorax and roentgenograms made, it is clearly seen that all the obscure densities in the thoracic roentgenogram are of visceral origin.

3. The vessels and adventitious connective tissue cast distinct shadows on the roentgenogram.

4. The shadows cast by the bronchi are linear and do not easily fuse to form a broad shadow such as that seen ordinarily in the hilus

region. On the other hand, the shadows of blood-vessels are uniform, and when filled with blood might easily fuse to form the greater part of the hilus shadow.

5. The lumen of the bronchus more than compensates for the fibrous tissue in its wall, so that the bronchial shadow consists (on the negative) of a dark band bounded by two narrow light bands, the latter being due to the bronchial wall in section.

6. The vessels correspond to the bronchi and are distributed in a similar manner throughout the lung, except the first one or two subdivisions at the hilus.

7. The hilus shadow is not due to lymph-nodes, although in some cases of disease these might participate in its formation.

Note: After writing the foregoing we discovered a reference to similar work done earlier by Dunham, Boardman and Wolman, who conclude that the hilus shadow is due jointly to bronchi and vessels; also work by Fraenkel and Lorez, who attribute the entire hilus shadow to the vessels, especially the pulmonary artery; and work by Sewell and Childs, who like us, believe that the hilus shadow is almost entirely due to the vessels.

No reference was made to any injections of citrated blood. For this reason, and also for the reason that the workers mentioned did not perfectly agree among themselves, the present report is published.

REFERENCES

1. Riviere: Early Diagnosis of Tubercle, Oxford University Press, London, 1914.

2. Dunham, Boardman and Wolman (Phipps Dispensary): The Stereoscopic X-Ray Examination of the Chest with Especial Reference to the Diagnosis of Pulmonary Tuberculosis, Bull. Johns Hopkins Hosp., 1911, xxii, 229.

3. Fraenkel, E., and Lorez, A.: The Anatomical Meani. _ of the Hilus Shadow in the Skiagram of the Chest, Arch. Roentgen Ray, 1910, xiv, 288.

4. Sewell, H., and Childs, S. B.: A Comparison of Physical Signs and X-Ray Pictures of the Chest in Early Stages of Tuberculosis, THE ARCHIVES INT. MED., 1912, x, 45.

METASTATIC CALCIFICATION *

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Metastatic calcification would seem to be a rare condition, if estimated by the number of cases reported in the literature, but it is undoubtedly often overlooked, and many cases that are recognized are probably not recorded. In 1911 I was able to collect from the literature but twenty-nine cases,[1] and since that time there have been but two more cases recorded (those of M. B. Schmidt[2] and Schober[3]) so far as I can find. The small number of cases published, and especially the important bearing this condition of metastatic calcification has on the general problems of calcium metabolism, ossification and pathological calcification, makes each additional case worthy of study and report.

By metastatic calcification is implied that the wide-spread deposition of calcium salts is a result of their excessive absorption from the normal depots, and excludes instances in which wide-spread tissue injury is the primary cause of a deposition of calcium salts. As Virchow inferred when describing this condition originally, it is a result of oversaturation of the blood with calcium salts. This view has received almost unanimous acceptance because it was found that metastatic calcification usually accompanied extensive destruction of bone tissue (for instance, tuberculosis or neoplasms of bones, and leukemia), which would result in the absorption of considerable amounts of the bone salts. This explanation received experimental support from Tanaka,[4] who injected soluble calcium salts into the peritoneal cavity of animals and found not only local deposits of calcium, but also deposits in remote tissues; his work has been corroborated by Katase,[5] who obtained the same results also by subcutaneous and intravenous injections.

One of the striking features of metastatic calcification as observed in human pathology is the predilection of the lungs, stomach and kidneys as the site of deposit. As the deposit, contrary to the rule in ordinary pathological calcification, takes place in tissues not previously

* Submitted for publication Oct. 16, 1914.
* From the Department of Pathology, University of Chicago.

2. Schmidt, M. B.: Deutsch. med. Wchnschr., 1913, xxxix, 59.
3. Schober: Inaugural Dissertation, Breslau, 1914; quoted by Stumpf, Centralbl. allg. Pathol., 1914, xxv, 801.
4. Tanaka: Biochem. Ztschr., 1911, xxxv, 113.
5. Katase: Beitr. pathol. Anat. (Ziegler's), 1914, lvii, 516.
1. Wells, H. G.: The Archives Int. Med., 1911, vii, 721.

injured or altered, and as these three organs are not often the site of calcification independent of local lesions, their selection is striking. The explanation would seem to lie in the fact that in these tissues we have the three chief places in the body where acids are excreted, and where, in consequence, the fluids must be most alkaline. An especially conclusive bit of evidence of the correctness of this hypothesis is furnished by metastatic calcification in the stomach, where, as pointed out by Hofmeister,[6] the deposits are limited to the interglandular tissue about the upper part of the glands of the fundus, that is, exactly corresponding to the location of the acid-secreting parietal cells.

In another place[1] I have pointed out the important part played by the carbon dioxid of the blood and tissue fluids in the transportation, absorption and deposition of calcium salts. Metastatic calcification provides a striking demonstration of this fact, not only by the marked and almost invariable calcification of the alveolar walls of the lung where the carbon dioxid is given off from the blood, but especially by those cases in which there is found also an extensive deposition of lime salts in the pulmonary veins, the left side of the heart, and the systemic arteries, that is, where the blood is poorest in carbon dioxid. Among such cases are the following:

Lazarus and Davidsohn[7] found in a 19-year-old girl with sarcoma of the dura eroding the skull, resulting in extensive metastatic calcification, a marked calcification of the intima of the left auricle, without involvement of the muscle itself. There was no calcification of the left ventricle or in the large pulmonary arteries and veins, and but a single calcified plaque in the aorta. It is particularly noted that the calcium has been deposited from the lumen of the auricle directly into the adjacent endocardium, supporting the hypothesis that the existing condition is the result of oversaturation with calcium from the blood in the left auricle.

Versé[8] described a case of myelogenous leukemia in a 25-year-old laborer, in which there was metastatic calcification that was especially marked in the lungs and left auricle. The latter is described as follows: The entire intima was white, except here and there some gray stripes and lines in places that were not calcified. The calcification ceased at the orifices of the pulmonary veins, extended down to the mitral ring and extended somewhat into the auricular appendix. The musculature of the heart was free from calcification. In the pulmonary artery there were only a few small yellow thickenings, but in the abdominal aorta were larger plaques with calcification, and also the media of the arteries of the lower extremities was much calcified. The lung contained numerous areas of calcification of the alveolar walls, and the pulmonary veins were heavily calcified with large rigid plaques. The deposits gave reactions for carbonates and phosphates. Microscopically the pulmonary veins showed the calcification to involve the media, but with the elastic fibers well preserved. In the auricle the deposits occupied the endocardial and subendocardial connective tissues, reaching to but not involving the muscle; here also the elastic fibers were not destroyed except in the oldest areas. In the kidneys the calcification was limited to a few areas in the cortex.

6. Hofmeister: Ergebn. d. Physiol., 1910, ix, 429.
7. Lazarus and Davidsohn: Ztschr. f. klin. Med., 1906, lx, 314.
8. Versé: Verhandl. d. deutsch. path. Gesellsch., 1910, xiv, 281.

M. B. Schmidt's[8] case is one of general calcification without bone destruction, and therefore not entirely within the strict interpretation of "metastatic calcification." Schmidt himself calls it *Kalkgicht*. Advanced nephritis was present in this as in most of the other cases of this sort, and is considered responsible, although the correctness of this assumption is questionable in view of the fact that the kidneys normally excrete but a small fraction of the calcium, most of which is eliminated through the bowels. There was an extensive calcification of the myocardium of the left ventricle, which contained 2.32 per cent. calcium oxid. It was especially located beneath the pericardium, and no mention is made of the condition of the left auricle. There was calcification in the lungs, kidneys, pulmonary veins, stomach, and especially in the systemic arteries. In some of the vessels there were free precipitates of lime salts independent of thrombi or infiltration of the vessel walls, which seem evidence that the deposition has been the result of an oversaturation of the blood with the salts.

It is interesting, also, to go back to the report of Küttner[9] in 1872 of a case of extensive bone disease with extreme calcification of all the systemic arteries, for this observation led Küttner to suggest for the first time the possible importance of the poverty of carbon dioxid in the arterial blood as the explanation of this particular localization, as well as the common occurrence of metastatic calcification in the lungs. In this case, however, there was no involvement of the heart recorded, so it does not fall into the group under discussion.

That calcification of the left auricle is common in metastatic calcification would seem probable, for we find it occasionally mentioned incidentally without particular attention being given to it. For example, Jadassohn,[10] in reporting a case of metastatic calcification resulting from osteomyelitis, merely mentions the presence in the endocardium of the left auricle of several hard white platelets, and of calcification of the papillary muscles and the large vessels in the lungs, but discusses chiefly the concomitant calcification of the skin. Also, in Schober's[3] case there was extensive calcification in the left auricle. Probably this endocardial and intimal localization has been overlooked or not mentioned by several of the observers of cases of metastatic calcification.

A case of typical metastatic calcification in myelogenous leukemia, with extensive involvement of the left side of the heart, thus resembling Versé's case, recently came under my observation in the necropsy room of the Cook County Hospital.

The patient was a Bulgarian laborer, 30 years old, who had a typical myelogenous leukemia of unknown duration. From the necropsy record, which shows the usual findings of myelogenous leukemia, the following points are extracted:

The heart was slightly increased in size by thickening of the left ventricle. There were a few small yellowish subpericardial areas which were not evidently calcified, but the coronary arteries showed extensive calcification. The foramen ovale was open, but protected by a valve-like flap, near which in the left auricle was a calcified plaque. Several other delicate calcific deposits appeared in the endocardium of the left auricle, involving about two-thirds of its entire

9. Küttner: Virchow's Arch. f. path. Anat., 1872, lv, 521.
10. Jadassohn: Arch. f. Dermatol., 1910, c. 317.

surface. Nothing of the kind could be found in the right auricle or ventricle, or in the pulmonary arteries. In the left ventricle under the endocardium there were light colored, irregular streaks and areas in the myocardium which grated when scraped with the knife. These calcified areas were quite numerous and distributed everywhere throughout the left ventricle, most numerous in the septum; but there were no such deposits found in the right ventricle.

The lungs did not collapse, showed a diffuse increase in consistency, and were somewhat dark in color. The cut surface was dry, slightly brownish, and semi-solidified. There was a slight grating sensation on cutting, and a sandy feel to some areas, especially where vessels were cut across.

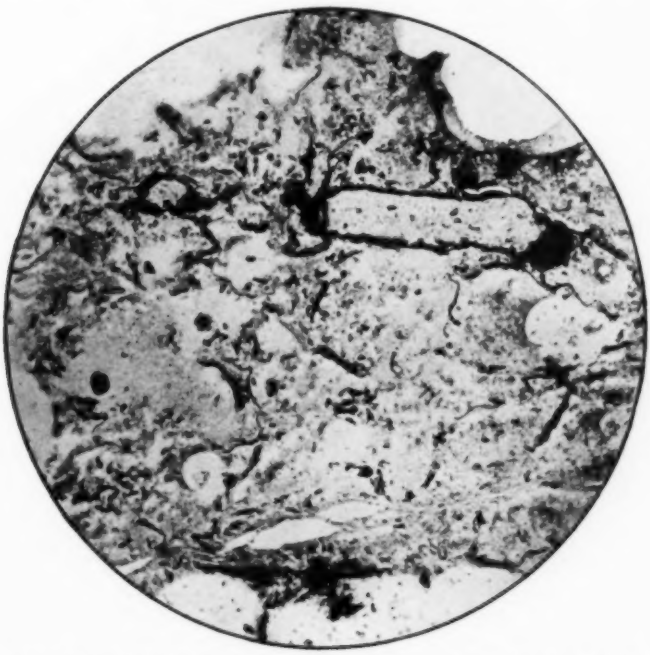

Fig. 1.—Lung, fixed in alcohol and stained by von Kossa's method with silver nitrate. The black-stained phosphate deposits are seen especially in the walls of two pulmonary veins, whereas a section of an artery nearby is not brought out because of the lack of calcification. The discontinuous calcium deposits in the alveolar walls are also well shown. Magnified 75 diameters.

All the other viscera showed the changes usual in leukemia, there being a marked leukemic infiltration of the liver and kidneys, without fibrotic changes in either. The marrow of the sternum and clavicle showed a marked hyperplasia, with destruction of bone tissue; in the sternum there were several well-defined yellow areas in the marrow. Other bones could not be examined, except the calvarium, which seemed to be normal.

No gross evidence of calcification was observed in the stomach or elsewhere in the alimentary tract. The blood-vessels in general were not calcified or sclerotic, this including the vessels of the brain.

Samples of lung tissue and heart muscle were preserved in alcohol. Unfortunately, the other tissues were preserved in Zenker's fluid containing acetic acid, and none of the stomach was kept.

Histological examination of the viscera in general showed only the usual changes of leukemia, there being no calcification demonstrable in Zenker's fixed tissues except in the lungs, myocardium and kidneys. Examination of alcohol-fixed tissues showed the following changes:

Lung (Fig. 1).—The heaviest deposits are in the large veins, occurring as thick, rather homogeneous masses which lie beneath the intima and often occupy

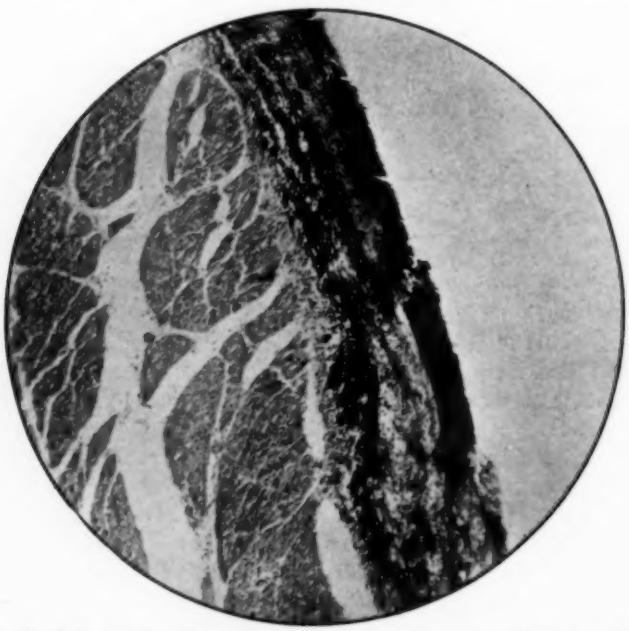

Fig. 2.—Left auricle. Stained by hematoxylin and eosin. The heavy deposit of calcium in the endocardium is shown without involvement of the underlying muscle. This illustrates the fact that the deposition comes from the blood passing through the left auricle. Magnified 52 diameters.

the entire media. The intima itself does not seem to contain any calcium. About some of the veins there is a heavy deposit in the adjacent alveoli, some of which are greatly thickened by masses of hematoxylin-stained homogeneous material. The adventitia of the vessels is not affected. Some of the larger bronchi show a heavy deposit, which is especially marked in the basement membrane beneath the epithelium, although in some places the muscularis is also involved. This finding is of interest in connection with Katase's statement that calcium is excreted through the bronchial mucosa. There is considerable calcification of the bronchial cartilages. The pulmonary arteries show no calcification and form a striking contrast to the adjacent calcified veins. In the alveolar walls there is considerable calcification, involving to a greater or less extent

nearly all the alveoli examined. Here the deposit occurs in a hyaline matrix, apparently formed by the basement membrane beneath the alveolar epithelium, and appears in the form of platelets or rods and scales. In addition to the calcification and a slight leukemic infiltration, there is a marked accumulation of pigmented, typial *Herzfehlerzellen*.

Left Auricle (Fig. 2).—Under the intima is a heavy layer of calcium salts, which is, however, discontinuous. It is deposited in narrow bands, which seem to correspond to fibers of the intimal tissue. In places where the process is early, at the margins of the densely calcified areas, it seems to be definitely beginning as a deposit in the elastic fibers, which are picked out as by a selective

Fig. 3.—Left ventricle. Stained with silver nitrate, showing the heavy deposits in the intima of the small branches of the coronary arteries, and also the heavy deposits within the muscles themselves. Magnified 60 diameters.

stain. It extends down to and in places slightly invades the muscular fibers where there seems to be some proliferative reaction to the deposits. The small arteries also show heavy deposits in the intima, which in some places slightly invade the media. There are no evidences of myocarditis or endarteritis outside the calcified areas; no deposits in the vein walls. A sympathetic ganglion in the pericardium shows no changes.

Left Ventricle (Fig. 3).—The heaviest deposits are again in the endocardium, where considerable areas show dense masses of calcium, often extending down into the underlying muscle. The arteries nearly all show heavy deposits in the intima, which usually is unaltered in all other respects; a few of the larger arteries show some proliferation of the intima where not calcified. Deep in

the myocardium are also calcium deposits, which are usually located in the vicinity of small arteries. Here the deposit is in the muscle fibers themselves, in early stages being in the form of small granules, in the later stages so dense that the structures are obliterated, sometimes with transverse lines of calcium, and often with fragmentation of the muscle fibers. In most instances there is no evidence that there has been any preceding change in the muscle fibers, but in some areas there is a concomitant local fibrosis, which might, however, be secondary to the calcification. There are no areas of leukemic infiltration. A large artery shows calcification selectively in the elastic lamina, with also a heavy deposit in the adventitia and in some foci in the media.

When the calcium is removed with acid and the section stained with hematoxylin and eosin, the muscle fibers that were calcified are found to be homogeneous, fragmented, but often with intact nuclei.

Kidney.—Besides extensive leukemic infiltration, there are numerous tubules showing calcification of their epithelium, and others showing calcified masses in the lumen. The glomerules and arteries are not calcified.

As the other tissues were not preserved in alcohol, but in Zenker's fluid, which dissolves out small deposits of calcium, we cannot be sure whether there was calcification in them or not. Nothing was found in the Zenker-fixed tissues that suggested the existence of calcification previously.

Sections through the sternum show the usual features of bone marrow in myelogenous leukemia, including an active destruction of the bone tissue.

Sections of the calcified tissues stained by von Kossa's silver nitrate method gave good selective staining of the deposits, showing that phosphates were present. Sections treated with hydrochloric acid gave a slight effervescence of gas showing the presence of carbonates. When treated with sulphuric acid the typical crystals of calcium sulphate were formed.

To recapitulate, we have here a typical case of metastatic calcification, resulting from bone destruction in myelogenous leukemia, making the thirty-second case of metastatic calcification found recorded. Of particular prominence and significance in this case are the heavy calcium deposits in the endocardium of the left side of the heart, the intima of the pulmonary veins and of the cardiac arteries, which illustrates as by a natural experiment the importance of the carbon dioxid of the blood in the transportation of calcium. When bone tissue is being rapidly absorbed, the venous blood becomes loaded with a greater amount of calcium (probably in the form of tribasic calcium carbonphosphate[1]) than arterial blood can hold in solution. Hence in this case of metastatic calcification no calcium is found in the right side of the heart or in the pulmonary arteries, but when the blood has passed through the lungs and lost a large part of its carbon dioxid, the calcium salts are precipitated in the pulmonary veins, the left heart and the arteries and taken up by the adjacent tissues.

STUDIES IN PANCREATIC DISEASE*

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PART I.—DUODENAL CONTENT ANALYSES AS AN INDEX OF DISEASE, AND FUNCTIONAL ACTIVITY OF THE PANCREAS

A reliable method for ascertaining the functional activity of the pancreas, has been eagerly sought for the last few decades, in fact since the time that Kuntzman, Richard Bright and Claude Bernard first called attention to this gland as a possible focus of disease.

The large variety of tests offered, some ten to twenty in number, is in itself testimony to the fact that no one test has met the mark or has satisfied the rigorous requirements of both pathologist and clinician. These tests fall into three groups: tests of the external secretion of the pancreas, usually carried out on the stool (Fuld-Gross), the urine (Wolgemuth), or gastric contents (Volhard oil test); tests of the internal secretion, consisting of tests for glycosuria, the Cammidge reaction, the Loewi pupillary dilatation test, etc.; and finally metabolism studies on the absorption of fat, nitrogen, etc., from the intestinal tract.

A detailed criticism of these many tests at this moment would be a large task, and one requiring unnecessary repetition of most valuable and thorough critical studies on the part of Frank,[1] Albu,[2] Werzberg,[3] Gross,[4] and very recently, Sladden.[5] It becomes increasingly evident, as one peruses the literature on pancreatic diagnosis, that there is little uniformity of opinion as to the value of the tests suggested, and general dissatisfaction expressed with practically each of them, most authors suggesting that more than one of these tests be per-

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* I take pleasure in acknowledging valuable suggestions and assistance on the part of Dr. S. Bookman. I am indebted for autopsy material and microscopic studies to Dr. F. S. Mandlebaum and Dr. William Thalhimer, and to the attending physicians and surgeons of the hospital for the privilege of studying the clinical material.

1. Frank: Arch. f. Verdauungskr., 1912, xviii, 121.
2. Albu: Samml. Zwangloser Abhand. a. d. Gebiet. d. Verdauungskr. u. Stoffwechselskr., 1911, iii, 1.
3. Werzberg: Arch. f. Verdauungskr., 1911, xvii, 533.
4. Gross: Ergebn. d. wissensch. Med., 1911, ii, 403.
5. Sladden: Quart. Jour. Med., 1914, vii, 455.

formed and an opinion formed from a general average of the results obtained.

It is difficult, for one not familiar with its intricacies, to appreciate how puzzling and complicated the problem is. Well authenticated cases are on record of the preservation of good health and nutrition with almost complete absence of the gland. On the other hand, severe nutritional disturbance and emaciation may be present in a gland with intact duct system and only a mild chronic inflammatory process taking place. Glycosuria as a symptom of pancreatic disease usually occurs late and with advanced destruction; yet it may be present with what is to-day considered a gland free of organic disease. The question of disturbances of metabolism and food absorption is a more puzzling one, there being even to this day no uniformity of opinion as to whether the external secretion of the pancreas is a prerequisite to normal food absorption and digestion.

The rapidly increasing knowledge of the importance of this organ both to the surgeon and the physician, and the realization of its frequent participation in diseases affecting the upper abdomen, have created a distinct demand for some reliable test that will give an indication of what is taking place in this inaccessible and deeply placed region.

Within the last four years several tubes or catheters have been devised for reaching the duodenum; these have afforded a means of aspirating the contents of this uppermost segment of the small intestine. As this novel means was quite evidently the most rational method of collecting for analysis the external secretion of the pancreas, it has been rather generally adopted. My own studies were begun four years ago, with the aim of demonstrating the presence of ferments in the duodenum, and of using the quantitative analysis of the strength of these enzymes as an index of the functional activity of the gland. In a preliminary paper[6] the question of technic was fully considered and the results of the first twenty-seven cases reported. From such a small series of cases definite conclusions were not possible, but sufficient encouragement was found in the analyses made, to offer the probability of a permanently reliable method. Since that time a larger series of over 120 patients has been studied; this series includes several varieties of pancreatic disease; in these cases additional chemical tests in intestinal absorption have been performed. Necropsies have been performed in a number of them, and in these the valuable opportunity was offered for comparing the existence and strength of the external secretion and the absorptive ability of the intestine for fat and

6. Crohn: Proc. Eighth Internat. Congress Applied Chem., 1912; Am. Jour. Med. Sc., 1913, cxlv, 393.

nitrogen; in addition the organic condition of the gland was definitely established.

This larger series of cases has afforded another and most valuable fact: one, indeed, quite indispensable to the method, namely, the existence of a normal standard of ferment strength for the external secretion of the pancreas. In the present series of 120 cases, only seventeen showed a deviation from the normal; of these we should exclude six instances in which complete absence of ferments existed, due to tumor obstruction of the ducts. In the remaining 103 cases, a uniform, or as Bondi[7] has called it, "a monotonously uniform" strength of ferments was found. We must accept the figures of these 103 cases as representing the activity of a normal pancreas. In reading the results of the chemical analysis, it is well to compare the activity of each ferment with the average strength of that enzyme as ascertained for normal cases. A general idea will then be had of the activity of the fluid obtained. This is superior to testing for only one ferment. Of the three enzymes calculated, lipase is undoubtedly the most variable, trypsin the most constant. By far the most reliable single index is the tryptic activity, as this protease is found uniformly when the ducts are patent. Normally, 10 c.c. of 0.1 per cent. casein solution is digested in twenty-four hours by duodenal contents in dilution of 1 : 3,000 to 1 : 10,000 or even higher dilutions.

The amount of duodenal contents collected cannot be utilized as an index of the amount of pancreatic juice secreted; some cases yield 4 to 5 c.c., some up to 15 to 20 c.c., during the five minutes of moderate continuous aspiration practiced. The strength of the ferments does not vary with the amount aspirated, the enzyme strength of over 90 per cent. of the cases being a constant one in spite of considerable variations in the amount of fluid obtained.

Before proceeding to discuss in detail the results obtained by me in duodenal content examinations, it would be advisable to review the results of similar investigators in the same field, as also the articles which have appeared since the preliminary publication of my first paper.

Einhorn,[8] in 1910, examined the duodenal contents in sixteen cases of various gastric disorders; seven of these cases were instances of achylia gastrica; ferments were present in practically all of these trials, trypsin twice being reported as absent. He further noted the absence of bile in a case of chronic icterus (exact diagnosis not stated).

7. Bondi: Arch. f. Verdauungskr., 1913, xix, 692.
8. Einhorn: Deutsch. med. Wchnschr., 1910, xxxvi, 1519; Jour. Am. Med. Assn., 1910, lv, 6.

Junghans[9] reported results with the Einhorn duodenal bucket (not catheter). In his examinations of fifty cases he succeeded in obtaining duodenal juice in 60 per cent. of his efforts; in a single instance only, one of pancreatitis with fatty stools, the ferments were absent. v. Barth Wehrenalp[10] was less successful with the duodenal bucket, allowing only three hours for the passage of the bucket through the pylorus and into the duodenum. Junghans left the bucket in over night. Their results, however, practically coincide.

Quantitative estimations of the pancreatic ferments were impossible on account of the small amount of fluid obtained with the string and bucket method.

Einhorn and Rosenbloom,[11] using for the first time the duodenal catheter and pump, found fairly constant strengths for the pancreatic ferments obtained; they were further able to demonstrate variations in the outflow of the pancreas, following injections of secretin, hydrochloric acid, sodium bicarbonate, broths, etc.

Crohn[6] reported on twenty-nine cases of various pathological diseases, and a study of a normal control. Constant figures for normal individuals and for persons not suffering from disease of the pancreas were obtained. A case of pancreatitis showed marked diminution of the ferments. Hypertrophic cirrhosis of the liver showed overactivity of the enzymes; in cases of diabetes mellitus the ferments were all present, amylase if anything, being exceedingly active.

Hess,[12] employing an ordinary soft rubber Nélaton catheter in children, demonstrated the presence of amylase, lipase and trypsin in the duodenal contents of new-born and older infants. Little difficulty was experienced in catheterizing the duodenum in a large series of cases. Hess asserts that in two cases of marasmus, paralytic hypersecretion was found; in seven cases of pylorospasm a true functional pancreatic hypersecretion was noted.

In a later paper, Hess[13] reported duodenal catheterization on infants suffering from acute diseases. He again found all the ferments constantly present; trypsin was noted as the most reliable ferment. In thirteen cases of acute intestinal intoxication the ferments were normally present except lipase, which was definitely diminished. Hess suggests that this may account for the poor tolerance of fats in these conditions.

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9. Junghans: Zentralbl. f. d. ges. Physiol. u. Path. d. Stoffwechs., 1911, vi, 49.
10. Von Barth Wehrenalp: Internat. Beitr. z. Path. u. Therap. d. Ernährungsstör ung., 1910, i, 530.
11. Einhorn and Rosenbloom: THE ARCHIVES INT. MED., 1910, vi, 666.
12. Hess: Am. Jour. Dis. Child., 1912, iv, 205.
13. Hess: Am. Jour. Dis. Child., 1913, v, 268.

Ewald, Magnus Levy and Lazarus spoke favorably of the method, though having no personal experience with it.

Oscar Gross[4] denounced the method as impracticable and dangerous, as did also Kleinberger.[14]

Frank[1] reported that he succeeded in recovering duodenal contents in 14 out of 24 cases (60 per cent.). Ferment findings were constant, though no exact quantitative estimations were attempted. In no case was blood ever aspirated. Frank asserts that where duodenal contents are obtained, one has absolutely the best criterion of the functional activity of the pancreas, particularly where quantitatively tested.

G. A. Friedman[15] obtained duodenal contents in all of eighteen cases; marked variations in the quantitative strengths of the ferments prevented him from drawing deductions.

In the last year four interesting reports have been published.

Chace and Myers[16] report on duodenal content examinations in thirty cases. No difficulty was encountered in obtaining the secretion in any of their cases. Active ferments were obtained in all instances, though considerable variation in the strength of these ferments was noted. In a case of hypertrophic liver cirrhosis the ferments were normal, as also in a case of pernicious anemia. In a case of chronic pancreatitis, enzymes, except for a trace of lipase, were absent from the duodenal contents. The variations in gastric acidity in no way influenced the strength of pancreatic enzymes.

Bondi and Solomon[17] never failed to obtain bile and pancreatic ferments in over 100 cases. The strength of the ferments was fairly uniform. In two clinically definite cases of chronic pancreatitis the ferments were absent. These are the only variations from the normal.

Bondi,[7] in a large series of cases, obtained duodenal contents in a fasting state, using water as a test meal. Large variations in the quantity of duodenal contents were found; the ferment strengths, however, are uniformly constant, variations in the three ferments being parallel.

In three cases of cholelithiasis the ferments were diminished. In a case of diabetes the ferments were diminished. Blood was occasionally found, but no significance is laid on this point.

In a recent paper by Einhorn,[18] twenty-four cases were studied. Of six cases of pancreatic lesions, four showed absence of one or two ferments; in two cases of pancreatic tumor, the ferments were present. Cloudy, turbid bile indicates gall-bladder or duct disease, in his opinion.

14. Kleinberger: Berl. klin. Wchnschr., 1909, xlvi, 2329.
15. Friedman, G. A.: Med. Rec., New York, 1912, lxxxi, 355.
16. Chace and Myers: THE ARCHIVES INT. MED., 1913, xii, 168.
17. Bondi and Solomon: Wien. med. Wchnschr., 1913, lxiii, 1722.
18. Einhorn: Am. Jour. Med. Sc., 1914, cxlviii, 490.

In general, one may say from the literature, that the Einhorn duodenal tube is the instrument of choice in duodenal instrumentation. With increasing experience, better results, including quantitative estimations, are noted. Fairly uniform figures for ferment strengths are obtained by all the authors. Pancreatic disease is the only one in which serious deviations from the normal occur.

REPORTS OF CASES

In my present series, consisting of over 120 cases, 103 cases are regarded as presenting normal pancreatic function; these include many pathological conditions; gastric lesions, benign and malignant, achylia gastrica, organic syphilis, exophthalmic goiter, secondary and primary anemias, malignant growths of various organs, diabetes mellitus, etc. As there is general uniformity of the ferment strengths in all these conditions, it is unnecessary to tabulate each of them separately.

Of the remaining seventeen cases, six presented complete absence of ferments in the duodenum; these are all cases of neoplastic obstruction of the ducts. The eleven other cases represent the instances of diminished pancreatic ferments, due to organic or functional pancreatic disturbance. By comparing the chemical findings of the duodenum with the clinical, operative and, where possible, necropsy data, we will be in a position to ascertain to what extent the results of analysis of duodenal ferments correspond to disease of the pancreas.

The cases may more favorably be divided into groups and separately discussed (Table 1).

GROUP A — CASES OF DIMINUTION OF FERMENTS ACCOMPANYING ORGANIC PANCREATIC DISEASE

Cases 1 to 6, inclusive.

CASE 1.—Acute suppurative pancreatitis, abscess of body and tail of pancreas. Note the marked diminution of ferments, trypsin and amylase being entirely absent.

CASES 2 AND 3.—These are both examples of combined chronic interlobular and intralobular pancreatitis. Both cases show markedly diminished ferments. Both cases at autopsy presented diffuse chronic inflammation of the gland, the cirrhotic tissue infiltrating about and also within the acini. In Case 3, there was, in addition, a small infiltrating carcinoma beginning about the bile duct, but not occluding the pancreatic passages.

CASE 4.—This is one of primary chronic pancreatitis, characterized by jaundice and abdominal pain. At operation, the head of the gland was markedly swollen; no stones were found. The duodenal ferments were only moderately diminished. The patient recovered after cholecystenterostomy.

CASES 5 AND 6.—These are two illustrations of pancreatitis occurring as a complication of gall-stones. Both cases at operation presented cholelithiasis and an enlarged swollen head of the pancreas. Duodenal ferments, in both instances, were definitely diminished.

TABLE 1.—Analysis of Duodenal Contents and Disease of the Pancreas

Case	Diagnosis	Jaundice	Duodenal Contents					Absorption Tests				Stool	
			Bile	Ferments	Amylase, C.c.	Trypsin, 1:—	Lipase, C.c.	Intake		Output (Stool)		Neutral Fat Globules	Undigested Muscle Fiber
								Fat, Gm.	Nitrogen, Gm.	Fat, Gm.	Nitrogen, Gm.		
1. A. B.	Acute pancreatitis; abscess of tail and body...	Slight	+	Very weak	0	0	1.8	101	....	9.5(9.4%)	.....	Absent	Absent
2. S. R.	Chronic pancreatitis; intralobular type.....	Marked	0	Weak	0	6,000	0	71.3	22.18	24.7(34.7%)	3.6(16.4%)	Present	Present
3. L. V.	Chronic pancreatitis; intralobular type.....	Marked	0	Weak	6	700	0	....	....	.....	.....	Absent	Absent
4. M. W.	Chronic pancreatitis	Moderate	+	Weak	0	3,000	2.7	....	....	.....	.....	Absent	Absent
5. R. M.	Subacute pancreatitis; cholelithiasis	Absent	+	Weak	0	3,000	0	....	....	.....	.....	Absent	Absent
6. H. D.	Chronic pancreatitis; cholelithiasis	Moderate	+	Weak	3	1,000	0	....	....	.....	.....	Absent	Absent
7. R. K.	Hepatic cirrhosis; abdominal adenitis	Moderate	+	Weak	0	5,000	0	118	18	57.6(51.3%)	3.4(18.7%)	Absent	Absent
8. A. B.	Gastric carcinoma; advanced stage	Absent	+	Weak	9	0	1.8	....	....	.....	.....	Absent	Absent
9. B. S.	Carcinoma of bile duct and liver	Marked	0	Weak	7	2,000	1.4	....	....	.....	.....	Absent	Absent
10. H. R.	Melanotic sarcomatosis..	Absent	+	Weak	0	3,000	2.5	....	....	.....	.....	Absent	Absent
11. M. F.	Addison's disease.....	Absent	+	Very weak	0	40	0.6	71.3	....	18.7(26.2%)	.....	Absent	Absent
12. L. J.	Carcinoma of papilla of Vater*	Marked	0	Normal	24.0	30,000	3.3	....	....	.....	.....	Absent	Absent
13. D. S.	Carcinoma of papilla of Vater*	Marked	0	Normal	12.0	4,300	1.2	....	....	.....	.....	Absent	Absent
14. A. B.	Chronic pancreatitis; cholithiasis	Absent	+	Normal	4.0	10,000	2.1	240	30	13.8(5.6%)	1.8(6%)	Absent	Absent
15. T. L.	Tumor of head of pancreas	Slight	+	Normal	24.0	20,000	1.5	....	....	.....	.....	Absent	Absent
16. M. D.	Carcinoma pancreas; obstructed ducts	Intense	0	Absent	0	0	0	124	20.1	35(28.2%)	2.2(11%)	Absent	Absent
17. L. K.	Carcinoma pancreas; obstructed ducts	Intense	0	Absent	0	0	0	80.9	11.8	48.4(60%)	1.86(15%)	Present	Present

* Intermittent obstruction of pancreatic duct; mild interlobular pancreatitis.

GROUP B — CASES OF DIMINISHED FERMENTS WITHOUT ORGANIC CHANGES IN THE PANCREAS

Cases 7 to 11, inclusive

CASE 7 is one of cirrhosis of the liver with moderate jaundice, frequent stools and fever. Pancreatic disease was indicated by diminished ferments in the duodenal contents and poor intestinal absorption, as shown by metabolism studies. At the autopsy several days later, besides the cirrhosis of the liver, large swollen lymph-nodes were found clustered about the head of the pancreas; the pancreas itself was normal grossly and microscopically.

It seems here that some obscure subacute inflammatory process, characterized by lymphadenitis, had taken place in the upper abdomen, centered about the head of this gland. There was a loss in the stool of over 50 per cent of the fat ingested; mechanical obstruction to the duct did not exist. In this case it must be presumed that the pancreas suffered from the disease going on about it; a functional disturbance is noted.

CASES 8, 9 and 10 are examples of tumors in the epigastric region not involving the pancreas, yet having an indirect effect on it by their presence, disturbing the functional activity of the pancreas. In twelve other cases of neoplasms situated variously in the body, this indirect disturbance of the pancreas was absent.

CASE 11 is one of Addison's disease; the diminution in ferments is extreme, this being again an instance of disturbed function from severe disease elsewhere in the body.

GROUP C — CASES OF NORMAL FERMENTS WITH DISEASE OF THE PANCREAS

Cases 12 to 15, inclusive.

CASES 12 and 13 are instances of carcinomata of the papilla of Vater which had caused temporary intermittent obstruction to the ducts and mild secondary pancreatitis. At the time of the examination, ulceration of the tumor into the lumen of the intestine had allowed the escape of pancreatic secretion. The ferments were found in normal concentration in spite of the fact that moderate interlobular inflammation had occurred. The tumor itself, though infiltrating slightly the head of the gland, caused no disturbance in its secretion; neither did the slight chronic inflammation give signs of decreased efficiency. These two cases contrast strongly with Cases 2 and 3, in which chronic inflammation of intra-acinar type caused real damage to the secreting parenchyma.

CASE 14 was one of cholelithiasis and subacute pancreatitis; though the head of the organ was swollen, the elaboration of ferments was in no way interfered with.

CASE 15 was one of tumor of the head of the pancreas not causing obstruction of the ducts. In spite of its presence, the ferments were quite normal.

GROUP D — CASES OF PANCREATIC NEOPLASMS WITH OBSTRUCTION OF THE DUCTS — FERMENTS ABSENT

Cases 16 and 17

There are cases of tumor obstruction of the duct of Wirsung with complete absence of the ferments in the duodenum.

DEDUCTIONS

The following deductions may be drawn from these cases:

1. Most cases of pancreatitis present deficient external pancreatic secretion.

2. Severe disease in a neighboring abdominal organ may cause diminution of the functional activity of the pancreas.

3. Mild interlobular pancreatitis may exist without diminishing the strength of the external secretion. This is in contrast with intra-acinar inflammation, which causes severe depression of functional activity.

4. Pancreatic new growths, which do not obstruct the duct, do not necessarily cause diminished secretary power.

The first conclusion, that diminished ferments accompany most instances of organic disease, is a logical one and is fully substantiated by the six instances quoted. Some confirmation can be found in the literature. Thus Junghans[9] found in a case of pancreatitis with fatty stools, absence of the ferments in the duodenum. Bondi and Solomon,[17] in two cases of pancreatitis, found identical results. Einhorn,[18] in six cases of pancreatic lesions, noted constant diminution of one or more ferments in the duodenal contents. Similar findings have frequently been noted in the ferment analysis of stools, by Orlowski,[19] Gross,[4] Lifschütz[20] and others. Matko[21] even goes so far as to describe functional hyperchylia, hypochylia and complete achylia pancreatica, on the basis of variations in the tryptic content of the stools.

Unfortunately, the stool analyses for ferments are entirely unreliable; this has been demonstrated by Pratt,[22] who, in four normal individuals, was unable to demonstrate trypsin; also by Tileston,[23] who seven different times examined in vain the feces of a normal individual, not being able to find a trace of trypsin. My own results previously published[6] also demonstrate the unreliability of stool ferment tests as an index of pancreatic functional activity. I there mentioned several instances of normal ferments in the duodenum, but with no trace of enzymes to be noted in the stool. This conclusively proves that, with an open duct and normal pancreatic secretion, the stools may still fail to show ferments.

The examination of ferments in the duodenal contents remains as the one reliable means of ascertaining the strength of the pancreatic ferments.

The second conclusion, that diminished enzymes in the pancreatic secretion may occur with severe disease elsewhere than in the pancreas, is a novel, yet not an unnatural one. For gastric acidity and the gastric ferments similarly vary with severe disease elsewhere in the body, as in pernicious anemia or carcinomatosis. Addison's disease, melanotic

19. Orlowski: Ztschr. klin. Med., 1912, lxxvi, 460.
20. Lifschütz: Arch. f. Verdauungskr., 1913, xix, 562.
21. Matko: Arch. f. Verdauungskr., 1913, xix, 663.
22. Pratt: Am. Jour. Med. Sc., 1912, cxliii, 314.
23. Tileston: Trans. Assn. Am. Phys., 1911, xxvi, 513; The Archives Int. Med., 1912, ix, 525.

sarcoma and general carcinoma are examples, in my series, of functional depression of pancreatic enzymes. Sladden,[5] in his excellent critical review of the diagnosis of pancreatic lesions, calls attention to the interdependence of the pancreas on other surrounding organs and observes that it is frequently affected by their diseases. Frank[1] noted a case of peritoneal tuberculosis in which a similar diminution of duodenal enzymes took place. It should not be gathered from these remarks, however, that this consecutive depression of ferments takes place often enough to be an important factor. It is an extremely unusual occurrence. In eighteen cases of abdominal carcinoma it was noted only three times (16 per cent.); it was seen altogether five times in over 120 cases of various diseases.

We may really say that diminution of ferments speaks strongly in favor of pancreatic disease; rarely the presence of severe disease elsewhere in the body may cause a similar finding. This latter fact is at present of more scientific interest than practical value, as it has not yet occurred where the diagnosis of a pancreatic lesion was at issue, but always as an accidental finding while studying large groups of diseases for possible points of interest.

The third conclusion, that pancreatitis, particularly of the chronic interlobular form, may exist without any change in the ferments, is merely corroboration of the fact that not every disturbance of this organ affects its external secretion, or is sufficiently advanced or destructive to cause damage. Clinical experience teaches that a small remnant of functionating gland may preserve metabolism as normally as the whole gland. A similar process of reasoning will explain the existence of normal ferments in the presence of moderately advanced disease, as in case of localized tumor at the head of the pancreas without diminution of the ferments. In this case the duct system was patent. Einhorn[18] had two similar instances in his series.

By comparing the enzyme activity of the external secretion in each case with the necropsy findings, a certain relation is noted between the amount of pancreatic tissue destroyed and the diminution of ferments in the duodenal contents. In Case 1, one of diffuse suppurative pancreatitis with destruction of about five-sixths of the gland tissue, only the faintest trace of ferment remains. In the two cases of chronic intra-acinar pancreatitis, the ferments were moderately diminished; microscopically, a diffuse intra-acinar and interacinar cirrhosis was seen, but a large amount of parenchyma was intact.

In the two cases of temporary obstruction of the excretory ducts by localized neoplasms with secondary pancreatitis of the inter-acinar type, the cirrhosis was slight; the ferments were normal. In these cases the connective tissue surrounded the lobules in larger areas,

sparing the inner secretory portion of the acini. In all four instances there were ample secretory cells intact, showing the definite zymogenic granulation of actively secreting cells.

There is therefore in general, a proportion between the anatomical condition and amount of surviving parenchyma on the one hand, and the enzyme activity of the external secretion on the other. The depression of ferments in cases of primary intralobular pancreatitis is distinctly greater than in those of interlobular inflammation secondary to duct obstruction or inflammation. From this it would seem probable that intralobular pancreatitis is a primary degeneration of the secreting cells, and that the connective tissue increase is only a replacement fibrosis. Interlobular pancreatitis is a connective tissue proliferation, dependent on excretory duct obstruction, inflammation, or vascular changes. The loss of secreting acini and cells is here the sequel of the duct disease.

SUMMARY

From the analysis of the external pancreatic secretion obtained by duodenal aspiration, we may say that:

1. Pancreatitis, particularly of the acute inflammatory, or of the chronic intra-acinar type, evidences itself by diminished ferments.

2. Severe abdominal disease may cause a functional hyposecretion of the pancreas.

3. Some cases of pancreatic disease, not associated with widespread destruction of the parenchyma, continue to furnish a secretion of normal enzyme activity.

4. A general gross proportion exists between the amount of surviving parenchyma and the ferment strength of the duodenal contents.

PART II.—OBSERVATIONS ON FAT AND NITROGEN METABOLISM IN DISEASES OF THE PANCREAS

Intimately associated with the question of the entry into the duodenum of the external secretion of the pancreas and the variations in enzyme strength in health and disease, is the subject of the influence of the pancreatic juice on the general metabolism, or in a more restricted sense, on the fat and nitrogen absorption from the intestine. For both animal metabolism studies and absorption observations in human pancreatic disease have failed to present satisfactory or acceptable conclusions on the simple issue, "Is the external secretion of the pancreas essential to good intestinal absorption?"

The failure to solve this problem seems to have rested on the inability to demonstrate conclusively in every instance, experimental and human, that the pancreatic secretion was entirely cut off from the intestine when the observer claimed it to be. Where good absorption

was retained after tying off ducts, the critic drew the inference that some accessory duct had been overlooked. Where the same occurrence took place in a clinical case, the diagnosis of the disease was questioned, or doubt was expressed that the pancreatic duct or ducts were entirely closed by the pathological process at the time of the metabolism study, the necropsy being held several weeks or months later.

The demonstration of the non-patency of the duct at the time of the experiment, has usually rested on the absence of trypsin in the feces, but as has been previously stated, this evidence is untrustworthy.

In the duodenal tube one has an undoubted method of obtaining the contents of the duodenum and of definitely determining both the state of patency of the ducts and the activity of the pancreatic outflow. With these data at hand, absorption experiments could simultaneously be carried on. If, in addition, the organic condition of the gland was ascertainable, we would be in possession of all the data necessary to demonstrate the effect of exclusion of or variations in, the external secretion of the gland on fat and nitrogen metabolism. This fortunate combination of data I have been able to obtain in several cases of human pancreatic disease; absorption experiments and enzyme studies were prosecuted hand in hand; autopsy and pathological reports are furnished in most instances.

Before recounting in detail my own results, let us review in brief the extensive and conflicting literature on the subject:

EXPERIMENTAL ANIMAL METABOLISM

The earliest experiments of Claude Bernard[24] consisted of injecting irritating substances into the excretory ducts of the dog's pancreas. By them he established the function of the gland in supplying enzymes to assist in the digestion of food substances. As a result of his experiments, several attempts were made to tie the ducts and note the effect on digestion and absorption; no definite results were obtained. Pawlow[25] noted the retention of good absorption even after the ducts were tied, and in the presence of atrophy of the gland. Von Mering and Minkowski[26] and Minkowski[27] alone, conducted a series of interesting experiments establishing the fact that excision of the gland led to diabetes and almost complete loss of the ability to absorb food from the intestine. Abelmann,[28] working under Minkowski, showed that, after

24. Bernard, Claude: Memories sur le pancréas, 1856.
25. Pawlow: Quoted from Oser, in Nothnagel's Encyclopedia of Practical Medicine, 1903.
26. Von Mering and Minkowski: Arch. exper. Path. u. Pharmakol., 1899, xxvi, 371.
27. Minkowski: Arch. exper. u. Pharmakol., 1905, liii, 331.
28. Abelmann: Ausnutzung der Nährungstoffe nach Pankreas-Extirpation. Dorpat, 1890.

total extirpation of the pancreas, fat absorption was nil, while nitrogen absorption varied from 30 to 80 per cent. After partial extirpation, absorption was much better (fat 31.5 per cent., emulsified fat [milk] up to 80 per cent.). Sandmeyer's[29] results with partial extirpation are similar to those of Abelmann. Hedon and Ville[30] demonstrated that, with a biliary fistula alone, the loss of lard in the feces was 55 per cent.; after biliary fistula and partial pancreatectomy the loss of fat rose to 90 per cent. Harley[31] extirpated all but about one-tenth of the tail. The loss of fat was 62.1 per cent., of nitrogen 82 per cent. Rosenberg,[32] on the contrary, found no disturbance of absorption when as little as one quarter of the gland was preserved. He succeeded in causing atrophy of the gland by cutting all the ducts, arteries and veins leading to it (except the main pancreaticoduodenal vessels), yet noted little disturbance of metabolism, though the atrophy was advanced. Zunz and Mayer[33] found little disturbance of absorption after tying the ducts, though atrophy resulted. When the atrophic remnant of gland was removed, however, complete loss of the power to absorb was noted.

Lombroso[34] made a complete study of this question with the following results:

1. After simple ligature of the ducts, fat excretion was 14 to 44 per cent.

2. After extirpation of most of the gland and transplantation of a viable fragment under the skin, fat excretion was 22 to 54 per cent.

3. Ligation of ducts, extirpation of atrophied remnant some weeks later, fat excretion 40, 99 and 113 per cent., respectively, in each of three dogs.

4. In a dog with a viable subcutaneous transplant, the transplant was extirpated. Fat excretion rose rapidly to 97.8, 105 and 96.6 per cent.

5. Wherever the surviving graft was well preserved, good absorption existed, though no ferments were entering the intestine. Lombroso suggested the possibility that the maintenance of good absorption, as long as some living pancreas existed somewhere in the body, was due to the vicarious absorption of the enzymes and resecretion of them through the bile into the intestinal tract. Yet he was never able to demonstrate increased ferments in the bile of such dogs, nor active

29. Sandmeyer: Ztschr. f. Biol., 1894, xxxi, 12.
30. Hedon and Ville: Compte rend. Soc. de biol., 1892, p. 308.
31. Harley: Jour. Path. and Bacteriol., 1895, iii, 245.
32. Rosenberg: Arch. f. d. ges. Physiol. (Pfluger's), 1898, lxx, 371.
33. Zunz and Mayer: Ref. Jahresb. u. d. Fortschr. d. Thierchem. (Maly's), 1905, xxxv, 489.
34. Lombroso: Arch. f. d. ges. Physiol. (Pfluger's), 1906, cxii, 531.

ferments in the intestine of dogs with ligated ducts. Nor was he able to improve the absorption of food by feeding duodenal juice of healthy animals to a dog with extirpated gland.

This painstaking work, as well as all that had gone before it, was criticized by Hess[35] and his pupil Sinn,[36] who pointed out that the pancreas of the dog contained more than one excretory passage, usually three or four; they said that Lombroso's results were due to his having failed to tie off all the ducts in his animals. In two dogs in which Hess tied the ducts he found excretion of fat, 46 per cent. and 95.3 per cent.

Lombroso[37] therefore repeated his experiments and arrived at substantially the same results. He further found that when a Pawlow permanent fistula was made and all the ferments excluded from the intestine, it made no difference in absorption whether the dog licked the outflow of the fistula or not; he concluded that intestinal absorption is independent of the external secretion, but is controlled by a ferment poured internally into the blood.

Burkhardt[38] duplicated Lombroso's experiments and came to diametrically opposite conclusions. When the dog licked the outflow of the fistula, absorption improved; when it was allowed to go to waste, absorption suffered.

Fleckseder[39] repeated these experiments with results similar to those of Lombroso, as did also Niemann.[40] In one of the dogs in which Niemann tied the duct and yet noted good absorption, he examined the duodenum for ferments, at death, but found none, proving that the duct was closed.

Jansen[41] demonstrated fair absorption of fats when an external pancreatic fistula existed; on removal of the graft piece by piece, the loss of fat increased rapidly up to 73.3 per cent.

Brugsch[42] in careful studies denied that absorption was dependent on the external secretion of the gland. He found but slight disturbance on tying the ducts, yet he also could demonstrate no ferments in the duodenum. He presumed the presence of an internal secretion or hormone which regulated absorption; he further attributed part of the loss to delayed motility in the intestine.

Still later work, however, again brings into doubt the conclusions of these various authors.

35. Hess: Arch. f. d. ges. Physiol. (Pfluger's), 1907, cxviii, 536.
36. Sinn: Dissert. Marburg, 1907, p. 29.
37. Lombroso: Arch. f. exper. Path. u. Therap., 1908-9, lx, 99.
38. Burckhardt: Arch. f. exper. Path. u. Pharmakol., 1908, lviii, 251.
39. Fleckseder: Arch. f. exper. u. Pharmakol., 1909, lix, 407.
40. Niemann: Ztschr. f. exper. Path. u. Therap., 1909, v, 466.
41. Jansen: Ztschr. f. physiol. Chem., 1911, lxxii, 158.
42. Brugsch: Ztschr. f. exper. Path. u. Therap., 1909, vi, 326.

Pratt, Lamson and Marks[43] seemed definitely to find in five dogs, deficient absorption of fat and nitrogen on ligation of all the ducts and insertion of omentum between the cut ends, nitrogen loss rising to 77.8 per cent., and fat loss to 88.7 to 95.2 per cent.

Benedict and Pratt,[44] feeding only meat to dogs with tied ducts, found nitrogen losses of 32.1 to 57.7 per cent.

It is most difficult to harmonize the various findings of these many investigators. One explanation alone will satisfy all the requirements: immediately on tying the ducts in animals, atrophy of the gland takes place. If all of the ducts are not ligated the atrophy does not occur. After ligature of the ducts, the resulting disturbance of absorption is dependent on the extent of atrophy resulting. When the atrophy is slight, the absorption remains good; when the gland deterioration is rapid, intestinal absorption is seriously altered. The experiments of Brugsch, Lombroso, Fleckseder and others are difficult to deny, and point definitely to the control of the absorptive processes by a hormone or internal secretion of the pancreas, and to a lesser degree to the direct action of the external secretion. Compensatory overactivity of gastric enzymes, intestinal erepsin, biliary lipase, etc., cannot explain the retention of good absorption with artificially obstructed ducts. Nor is the question answered by suggesting that the ferments are absorbed into the blood from the stagnant contents of the obstructed ducts, and reexcreted through the intestinal wall, for no increase or even maintenance of the usual ferments in the lumen of the intestine has ever been demonstrated.

OBSERVATIONS ON ABSORPTION EXPERIMENTS IN HUMAN PANCREATIC DISEASE

Even less conclusive are the results of absorption experiments in persons suffering from disease of this gland.[45]

Disturbances of fat metabolism in diseases of the pancreas were first observed by Kuntzman,[46] and independently by Richard Bright.[47] The latter's own words best describe the symptom of "fatty stool."

43. Pratt, Lamson and Marks: Tr. Assn. Am. Phys., 1909, xxiv, 266.

44. Benedict and Pratt: Jour. Biol. Chem., 1913, xv, 1.

45. In studying fat and nitrogen metabolism in pancreatic disease, it is essential to restrict oneself to cases in which the intake of food was carefully analyzed, as well as the output of feces. Only in this way can conclusions be reached, since analyses merely of stools, or general observations on the character, number, or consistency of the movements, without taking into consideration the nutritive value of the diet, are fallacious.

46. Kuntzmann: Jour. d. Pract. Heilk., 1824, lix, 45.

47. Bright, Richard: Medico-Chirurg. Trans., 1833, xviii, 1.

The condition to which I refer is a peculiar condition of the alvine evacuation, a portion more or less considerable assuming the character of an oily substance resembling fat, which either passes separately from the bowel, or soon divides itself from the general mass and lies on the surface, sometimes forming a thick crust, particularly about the edges of the vessel [steatorrhea].

The inconstancy of the symptom and the impossibility of harmonizing it with the pathological conditions were immediately recognized by Bright. For the symptom was present in three cases of carcinoma of the duodenum and head of the pancreas, but was absent in a case of cancer of the pancreas and duodenum in which the ducts were obstructed; also absent in two cases of cancer actually invading the gland, in one of which cases the duct was blocked at the head and enormously dilated.

Some years later Fles[48] made his remarkable observation on the large amount of muscle fibers remaining in the feces in patients suffering from pancreatic disease and Harley[21] and Le Nobel,[49] and Abelmann[28] noted in animals the increase in nitrogen excretion in similar diseases (azotorrhea).

Clinical observations in the next decades were numerous, but threw no further light on the interesting symptoms. Stimulated by the careful animal experiments of von Mering and Minkowski, accurate chemical observations were begun in 1895; since then the reports of carefully analyzed cases, while numerous, yet do not number more than forty examples.

To avoid monotonous repetition, the cases in the literature are classified and presented in Tables 2, 3 and 4:

TABLE 2.—Fat and Nitrogen Excretion with Closed Pancreatic Duct

Author	Fat Excretion,* Per Cent.	Nitrogen Excretion,* Per Cent.
Brugsch[42]	70	25.4
Harley[21] (inflammatory stricture)	73	40.0
Pratt[22]	58.9	50.9
Peripancreatic cyst, Delfino[72]	19.7	
Pancreatic abscess, Umber and Brugsch[37]	59.7	

*Represents per cent. of ingested fat or nitrogen, which was excreted.

48. Fles: Holland Arch., 1864, ii, 187 (quoted from Robson and Cammidge, "The Pancreas, Its Surgery and Pathology," 1907).

49. Le Nobel: Jahresb. u. d. Fortschr. d. Thierchem. (Maly's), 1886, p. 449.

72. Delfino: Deutsche Ztschr. f. Chir., 1913, cxxi, 280.

TABLE 3.—Fat and Nitrogen Excretion in Chronic Pancreatitis

Author	Jaundice Present	Fat Excretion,* Per Cent.	Nitrogen Excretion,* Per Cent.
Weintraud[73] (hepatic cirrhosis?)..	0	18	46
Weintraud[73] (atrophy of pancreas)	0	22	45
Salomon[75]	+	51	69
Glaessner and Sigel[61]	0	56.1	41.5
Brugsch and Koenig[74]	+	59	
Gigon[75] (calculus cirrhosis).....	0	20.5	43
Hirschfeld[58] (six cases).....	0	29.4-47.2	30.4
Keuthe[63] (calculi, atrophy).....	0	9.8	
Ehrmann[71] (after cholecystenterostomy).....	0	50.1	42.8
Tileston[23]	0	72	62
Barbour[76] (calculi)	0	.....	44.2
O. Gross[54]	0	55.4	50.8
O. Gross (calculi, atrophy).....	0	26.2	31
Ehrmann and Krüspe[77] (cholecystenterostomy)	0	33.3	34

* Per cent ingested fat or nitrogen.

TABLE 4.—Fat and Nitrogen Excretion with Tumors of Pancreas

Author	Obstructive Jaundice	Fat Excretion,* Per Cent.	Nitrogen Excretion,* Per Cent.
Deucher[78]	Slight	83	30
Deucher[78] (cholecystenterostomy).	0	52.6	19
Brugsch[42]	+	85	39
Brugsch[42]	+	64	20
E. Meyer[79] (carcinoma of head)...	+	38	34-41
Adler and Milchner[59].....	+	18	
Wynhausen[80]	.....	73.6	32.9
Brugsch[81] (ducts closed).....	+	60	36
Albu[2] (cancer and atrophy).....	+	79	
Tileston[20]	+	75.6	19.8
Tileston[23]	+	68	
Tileston[23]	+	52.6	14.5
Tileston[23]	+	45.6	21.1
Tileston[23]	+	49.1	
Pratt[22]	0	79.9	34.8

* Per cent. of ingested fat or nitrogen.

73. Weintraud: Die Heilkunde, 1898, iii, 67.
74. Brugsch and Konig: Berl. klin. Wchnschr., 1905, xlii, 1605.
75. Gigon: Ztschr. f. klin. Med., 1907, lxiii, 420.
76. Barbour: The Archives Int. Med., 1911, viii, 662.
77. Ehrmann and Krüspe: Ztschr. f. klin. Med., 1913, lxxviii, 122.
78. Deucher: Cor.-Bl. f. schweiz. Aerzte, 1898, xxviii, 321.
79. Meyer, E.: Ztschr. f. exper. Path. u. therap., 1906, iii, 58.
80. Wynhausen: Berl. klin. Wchnschr., 1909, xlvi, 1406.
81. Brugsch: Ztschr. f. klin. Med., 1905, lviii, 518.

Before critically analyzing these tabulations, it is essential again to recall that in most of the cases no mention is made of the patency of the pancreatic duct; further, many of the cases were not observed at autopsy, the clinical diagnosis alone determining the nature of the malady. Even autopsy descriptions often fail to detail the condition of the gland, and microscopic studies are very rare.

From these tables, however, we may note the following general averages:

Simple closure of the pancreatic duct, nitrogen loss, 38.8 per cent.; fat loss, 67.3 per cent.; acute pancreatitis abscess, nitrogen loss, 21 per cent.; fat loss, 59.7 per cent.; chronic pancreatitis, nitrogen loss, 46.3 per cent.; fat loss, 39.3 per cent.; tumors of pancreas, nitrogen loss, 28.6 per cent.; fat loss, 62.3 per cent.; pancreatic cyst, fat loss, 19.7 per cent. The figures for the normal excretion of fat and nitrogen, as given by Schmidt and Strassburger[50] are, nitrogen, 4 to 6 per cent.; fat, 12 to 16 per cent. In simple obstructive jaundice, the fat excretion increases to 20 to 30 per cent.; nitrogen absorption is practically unaffected.

Conclusions from these tables of absorption tests in man are most difficult, for all the cases were instances of advanced disease of the gland, sometimes associated with closure of the duct, and always with advanced disorganization of the parenchyma. Early examples of the disease and mild cases do not appear, as the clinical diagnosis in such cases before the appearance of the typical stool changes is never made.

From Table 5 we observe that simple benign cyst of the pancreas causes little disturbance of absorption; acute and chronic pancreatitis give rise to moderately severe disturbance, the nitrogen excretion rising to 21 to 46.3 per cent., and fat excretion to 39.3 to 59.7 per cent. Malignant neoplasms demonstrate a higher fat loss amounting to an average of 62.3 per cent., individual variations ranging from 30 to 83 per cent.

To the tabulated instances in the literature, I am able to add seven examples of careful absorption experiments in diseases of the pancreas. In all of these cases, duodenal contents obtained at the moment of the chemical studies, definitely determined the patency or non-patency of the pancreatic and bile ducts, as also the condition of the pancreatic external secretion. Careful autopsy studies accompany many of the cases.

TECHNIC

As an index of pancreatic metabolism, fat and nitrogen absorption tests were chosen. Though Fr. Müller[51] held that the poor absorption of these substances was due to poor digestion and preparation of the food in the intestine, as evidenced by diminished saponification, later observers have not confirmed this contention. Brugsch[42] experimentally

50. Schmidt and Strassburger: Die Feces des Menschen, Berlin, 1905.
51. Müller, Fr.: Ztschr. f. klin. Med., 1887, xii, 45.

demonstrated normal protein digestion and amino-acid formation in dogs with ligated ducts, and numerous clinical observers have frequently shown normal fat splitting and saponification in the intestine of human instances of pancreatic diseases (Tileston,[23] Pratt[22] and others). In later years, therefore, most workers have limited themselves to ascertaining absorption of food products; the claims of Bondi and Bondi,[52] that epithelial degeneration took place in the mucosa of the intestinal tract has found no confirmation and few believers. Nor will interference with intestinal and gastric motility account for the symptom of disturbed nutritional equilibrium as suggested by von Noorden[53] and by Brugsch.[42]

The diet employed for absorption experiments consisted of the following: Milk, 1,500 to 2,000 c.c. a day; eggs, three a day; bread, 100 gm. daily; cereal, 100 to 200 gm.; meat, about 50 gm., with fat, daily; water, normal amounts. The diet was varied in amount to suit individual requirements, some patients being unable to eat all that was tendered to them; however, when possible, uniformity in the amount and character of the food was preserved. The diet has a daily equivalent of about 120 gm. of fat, and 15 gm. of nitrogen. Gross[54] has shown that absorption varies very little with the quantity of food given, practically the same percentage of fat and nitrogen being absorbed with large variations in the food intake.

The absorption test was begun by the administration to the patient of a low saline enema, the return of which was discarded. The designated diet was then begun, a complete duplicate diet being sent to the laboratory for analysis. The diet and the collection of specimens of the stool were continued for two days, at the end of which period another enema was given. This enema was added to the collection of feces, the whole being sent to the laboratory. On receipt in the laboratory, macroscopic and microscopic examinations were made on the stool; the food, after the addition of alcohol, was dried to approximately constant weight over the water-bath. The stool was similarly dried; both feces and food were then ground in a mortar and pulverized. When the stool was too oleaginous for grinding it was first frozen, then ground. Nitrogen values were ascertained by means of the Kjeldahl process; fat by the Kumagawa-Suto[55] method.

In most of the cases in this series, one or two intestinal movements a day was the rule; more frequent evacuations were very exceptional. In no case was organic intestinal disease present, nor was intestinal or abdominal tuberculosis a factor. Both these latter conditions have been demonstrated by Fr. Müller[51] to cause disturbed absorption. Jaundice was present in most of my cases, usually a complete obstruction of the bile duct. To establish definitely a figure for absorption in cases of complete biliary obstruction, a preliminary test was carried out on a case of carcinoma of the bile duct with total occlusion of bile, but with

52. Bondi, S., and Bondi, J.: Ztschr. f. exper. Path. u. Therap., 1909, vi, 254.
53. Von Noorden: Handb. d. Path. d. Stoffwechsel., 1906.
54. Gross, O.: Deutsch. Arch. f. klin. Med., 1912, cvii, 106.
55. Kumagawa-Suto: Biochem. Ztschr., 1908, viii, 252.

normal pancreatic function and demonstrated by the duodenal tube. The excretion of fat was 32 per cent.; of nitrogen 6.6 per cent. This may be accepted as a control figure for a case of complete acholia. The difference between this figure and higher figures in the following series, would represent the pancreatic factor.

The results of the absorption tests on my series of pancreatic diseases is as shown in Table 5:

TABLE 5.—Absorption Tests*

Case	Diagnosis	Jaundice	Duodenal Contents		Intake		Output	
			Bile	Ferments	Fat	Nitrogen	Fat	Nitrogen
1. A. B.	Acute pancreatitis; abscess	Slight	+	Very weak	101	....	9.5(9.4%)	
	Group 2							
2. S. R.	Chronic pancreatitis, intralobular	Marked	0	Weak	71.3	22.18	24.7(34.7%)	3.6(16.4%)
3. A. B.	Chronic pancreatitis, cholelithiasis	Absent	+	Normal	240.0	30.0	13.8(5.6%)	1.8(6 %)
	Group 3							
4. R.K.	Hepatic cirrhosis; abdominal adenitis	Moderate	+	Weak	118	18	57.6(51.3%)	3.4(18.7%)
5. M. F.	Addison's disease..	Absent	+	Very weak	71.3	....	18.7(26.2%)	
	Group 4							
6. M.D.	Carcinoma of pancreas	Intense	0	Absent	124	20.1	35 (28.2%)	2.2 (11%)
7. L. K.	Carcinoma of pancreas	Intense	0	Absent	80.9	11.8	48.4(60 %)	1.86 (15%)

*This table is a rearrangement of the cases in Table 1.

Group 1. Fat absorption studies in a case of acute suppurative pancreatitis with severe diminution of all the ferments (very slight jaundice) demonstrated that on an intake of 101 gm. of fat, 9.5 gm., or 9.4 per cent., were excreted from the bowel. Of the excreted fat, 42 per cent. was neutral, 58 per cent. was split (fatty acids and soaps).

At the necropsy the body and tail of the gland were found to be entirely destroyed, only the head and a small portion about the duodenum being preserved. The duct of Wirsung was patent.

In this case, in spite of marked destruction of the gland, and extensive diminution of the ferments in the external secretion, the absorption of fat was excellent, thereby demonstrating that a small portion of intact pancreas can maintain absorption equilibrium, *even when the enzymes excreted into the intestine are markedly diminished.* In the stool no evidence of neutral fat was observed.

Group 2 comprises two cases of chronic pancreatitis (Cases 2 and 3, Table 5). Case 2 is one of moderately severe intralobular inflammation, causing, in addition, stricture of the bile duct (duodenal tube and necropsy). At the time of the test the ferments were markedly diminished, and bile was absent. The stools contained a few neutral fat globules and undigested muscle fibers. Absorption experiments demonstrated that on an intake of 71.3 gm. of fat and 22.18 gm. nitrogen, there was a fecal loss of 34.7 per cent. fat, and 16.4 per cent. of the ingested nitrogen.

Case 3 was one of mild pancreatitis with swelling of the head of the gland as shown at the operation. At the time of the metabolism test the duodenal tube demonstrated normal enzymes and normal quantity of bile. On an intake of 240 gm. of fat and 30 gm. nitrogen there was an excretion of 5.6 per cent. fat and 6 per cent. nitrogen; in other words, normal conditions.

Group 3 entails the discussion of two cases of functional depression of the pancreas caused by severe disease elsewhere in the body (Cases 4 and 5, Table 5). Both cases showed diminution in the enzyme activity of the duodenal contents; normal amounts of bile were entering the intestine.

Case 4 was one of cirrhosis of the liver with abdominal lymphadenitis; the parenchyma of the gland was microscopically and macroscopically normal. Absorption tests demonstrated that on an intake of 118 gm. fat and 18 gm. nitrogen there was an excretion of 51.3 per cent. fat and 18.7 per cent. nitrogen.

Case 5 was one of Addison's disease (clinical diagnosis). At the time of the test, duodenal ferments were definitely diminished; bile was abundant. On an intake of 71.3 gm. fat, 26.2 per cent. was excreted.

These two cases demonstrate that functional depression of the pancreas causes definite and marked interference with the absorption of food, and this, too, be it remembered, without evidence of organic disease of the secretory cells.

Group 4 comprises two cases of complete obstruction of the pancreatic duct by neoplasms. Complete biliary occlusion existed in both instances. Case 6 was observed in the first week of illness. On an intake of 124 gm. fat and 20 gm. nitrogen there was an excretion of 28.2 per cent. fat and 11 per cent. nitrogen. The complete acholia would account for the loss of 28 per cent. fat; the slight increase of nitrogen loss to 11 per cent. is the only result of complete absence of

ferments of the pancreas in the intestine.[56] As this patient was examined in the first week of symptoms, it may be presumed that the body of the gland was still intact, as innumerable animal experiments show that the process of atrophy which results on closure of the duct is a gradual one, taking time for its full development. Neutral fat globules and undigested muscle fibers were absent from the stool.

Case 7 (Table 5) differs from the former in that it was seen only after the malignant process had been in existence over three months. The duodenal tube demonstrated, at the time of the absorption experiments, complete closure of the bile and pancreatic ducts. Neutral fat globules and undigested muscle fibers appeared in the stool two weeks after the period of the absorption tests. On an intake of 80.9 gm. fat and 11.8 gm. nitrogen, there was an excretion of 60 per cent. fat and 15 per cent. nitrogen. The large loss of fat and protein was accounted for by the autopsy report; complete infiltration of all the pancreas by invading carcinoma; the portions of the parenchyma not actually destroyed by the new growth were atrophic and replaced by cirrhotic connective tissue.,

Three main conclusions may be drawn from these absorption tests in pancreatic diseases:

1. The nature of the disease does not determine the degree of fat and nitrogen loss.

2. The amount of food absorption is independent of the patency of the duct, or the activity of the enzymes of the external secretion of the gland.

3. The degree of interference with intestinal absorption is dependent on organic disease or functional derangement of the parenchyma of the gland, the pancreas probably normally controlling intestinal absorption by an internal secretion or hormone.

In substantiation of the first conclusion we may point to the wide divergence between the figures for absorption in the several diseases. In acute suppurative pancreatitis, for instance, the fat loss in Case 1 of my series is 9.4 per cent.; in a case reported by Umber and

56. It might be suggested in this case, that a patent accessory duct of Santorini supplied ferments to the intestine. Careful dissection in six cases of obstruction of the duct of Wirsung failed to show any instance of a patent accessory duct. Nor have any of the observers of pancreatic disease noted a patent accessory duct, except Tileston[23] who once found it. However, the duodenal tube, lying deep in the intestine, would not differentiate between main and accessory ducts; if ferments are being secreted it would discover them. When no ferments are found, it is safe to assume that all ducts are closed, as the Santorini duct in man opens with, or within a centimeter or two of the Vaterian papilla. At autopsy the accessory duct has often been seen, but never patent through to the lumen of the intestine.

Brugsch[57] (Table 2), this same loss was 59.7 per cent., or five times as great. In Cases 2 and 3 of my series the fat loss varied from 5.6 per cent. to 34.7 per cent., and the nitrogen loss from 6 per cent. to 16.4 per cent. A glance at Table 3 of the literature will demonstrate still greater variations.

In two cases of obstructing pancreatic tumors in my series, Case 6 shows the moderate losses of 28 per cent. fat and 11 per cent. nitrogen, while Case 7 presents a loss of 60 per cent. fat (more than double) and 15 per cent. nitrogen. A glance at Table 4 of the literature brings to light still greater divergences.

It is then evident that the nature of the disease plays a small rôle in determining the degree of food loss.

In substantiation of the second deduction, that the external secretion of the pancreas influences food absorption in only a small way, we note that absorption is equally good in Case 1 with almost completely absent ferments, Case 6 with no ferments at all in the intestinal tract, and Case 3 with normally active enzymes being secreted. Absorption is almost identical in all three instances in spite of the great divergence in the condition of the ferments.

Against the deduction that absorption is little disturbed by diseases depressing the enzyme secretion of the pancreas, may be offered the argument that compensatory overactivity of the gastric, salivary, or intestinal ferments (erepsin) may substitute itself for the former diminution (Meltzer, "Factors of Safety," in discussion of Tileston's paper[23]). But if we refer back to the section of this article on Experimental Pancreatic Disease, we will recall that all attempts to demonstrate such compensatory overactivity were futile. Failure also attended efforts to demonstrate absorption of stagnant ferment from obstructed ducts and reexcretion through the intestinal wall into the lumen of the gut. Experimentally, when the ducts are tied, trypsin is absent from the intestine (Brugsch,[42] Werzberg,[3] Lombroso[34]). The same fact holds good for human cases of obstructed ducts (Orlowski,[19] Gross,[4] Pratt,[22] Tileston,[23] Crohn,[6] Matko[21] and others).

After consideration of my own cases and of the experimental work the evidence fails to show that the external secretion of the pancreas is essential to good absorption.

In substantiation of the third deduction, that the degree of interference with absorption depends on organic or functional disturbances of the secretory parenchyma of the gland, we may bring the following evidence:

Of the two cases of pancreatitis in Group 2 (Table 5), in Case 2 the process was severe, general throughout the gland, intralobular in

57. Umber and Brugsch: Arch. f. exper. Path. u. Pharmakol., 1906, lv, 164.

form; every lobule had been attacked, within and without, by connective tissue, as shown by the autopsy. The absorption test showed a loss of 34.7 per cent. fat and 16.4 per cent. nitrogen. In Case 3, with swelling only of the head of the gland, relieved by operation, absorption was quite normal. Of the two cases of neoplastic obstruction of the ducts (Cases 6 and 7), absorption in the one examined early was still very good; in the patient examined late in the course of the disease (complete infiltration and atrophy of acini), absorption had suffered greatly.

Confirmation of this contention from the literature is difficult, as so few detailed necropsy reports exist, and the extent of destruction is so rarely mentioned. Moreover, the literature offers only such cases as demonstrated clinically free neutral fat or undigested muscle fibers in the stools or had the numerous, bulky, oleaginous evacuations typical of pancreatic disease. As this symptom, in my experience and in that of others, is a late one to appear, and indicates advanced disease, we naturally expect to find marked interference with absorption. Exact chemical methods in all these published cases do show severe losses in the food absorption.

If, on the other hand, we attribute the degree of food loss to the nature of the attacking disease, we find no consistent ground for such a classification. Pancreatitis cases vary in their fat losses between 9 per cent. and 72 per cent.; tumor cases vary between 18 per cent. and 85 per cent., or practically identical limits. Nitrogen losses vary similarly. The explanation is not to be sought in the nature of the malady, but in the amount of tissue destruction or functional disarrangement brought about within the gland. Thus, every case must be judged by itself, and the disturbance of gland function estimated by the degree of unabsorbed food.

Could one demonstrate a direct proportion between absorption interference and parenchyma disorganization, the problem would be solved. But the exceptions to such a rule are too numerous. Richard Bright noted this when he made his first observations on fatty stools. Franke[58] published three cases of complete excision of the pancreas for cancer without the development of glycosuria or fatty stools (one case observed for five months). Adler and Milchner[59] observed a case of abdominal tumor, with severe glycosuria and jaundice, yet excreting only 18 per cent. of the ingested fat. Walker[60] reports the case of a man observed for twenty-six years, who at necropsy showed pancreatic calculi blocking the duct and advanced atrophy of the parenchyma.

58. Franke: Arch. f. klin. Chir., 1901, lxiv, 364.
59. Adler and Milchner: Berl. klin. Wchnschr., 1908, xlv, 1487.
60. Walker: Med. Chir. Tr., 1889, lxxii, 257.

yet never developed glycosuria or fatty stools or other evidence of disease of the gland.

The most flagrant exception to the rule is the case reported by Glaessner and Sigel,[61] one of chronic pancreatitis. In this case there was a loss of 56.1 per cent. fat and 41.5 per cent. nitrogen. Five years later, Keuthe[62] performed absorption experiments on this same individual and discovered normal metabolism. At the autopsy (death due to pulmonary tuberculosis) pancreatic calculi and complete atrophy of the gland were found. No accessory pancreas was discovered.

These exceptional instances can be explained on only one common basis, namely, that small remnants of surviving parenchyma *can* and do preserve normal metabolism. But there is another factor besides the amount and size of the surviving fragment, and that is the functional capability of this remnant. This point is amply verified by the experiments of Lombroso, Fleckseder, Niemann and others who demonstrated nutritional equilibrium when a minute transplant of pancreas remained viable under the skin.

But if a small fragment of secreting tissue can maintain good absorption, why do we constantly observe severe disturbances in metabolism when the gland is largely preserved, showing only a moderate degree of chronic intra-acinar inflammation, but with large areas of well preserved and apparently actively secreting zymogenic cells? This query brings the same answer, that *not the amount but the functional activity of the surviving cells* determines the degree of balance or loss of equilibrium. It may be that chronic intra-acinar pancreatitis is, as suggested in an earlier paragraph, a primary degeneration of the secretory cells, not visible with our present microscopic methods, and involving that internal secretion of the gland which controls absorption, and so often carbohydrate metabolism. In that event the connective tissue would be only a replacement fibrosis, of no importance as an index of the severity of the disease.

That functional deterioration of the *external* pancreatic secretion may occur without organic disease was shown by the enzyme studies in Part 1 of this paper (Cases 7-11, Table 1). A similar functional deterioration may affect the *internal* secretion of the gland, resulting in disturbed absorption (Cases 4 and 5, Table 5). The present conception of the mechanism of the production of diabetes mellitus is founded on the idea of a similar disturbance to an internal secretion of the pancreas; here, too, it has frequently been impossible to demonstrate an organic lesion to the gland, even with the finest methods. Organic disease, sufficiently advanced to cause both diabetes and dis-

61. Glaessner and Sigel: Berl. klin. Wchnschr., 1904, xli, 440.
62. Keuthe: Berl. klin. Wchnschr., 1909, xlvi, 47.

turbed metabolism, may exist, though this is rare. Such cases were clearly described by Hirschfeld[63] in 1891. Fortunately, coexistent disturbance of both the internal secretions of the pancreas is unusual; fatty stools in diabetes are exceedingly rare, and glycosuria as a symptom of pancreatitis occurs only in 38 per cent. of cases according to Fitz,[64] and only 5.3 per cent. as given by Deaver.[65] The two internal secretions of the pancreas are separate entities, sometimes, but not usually, being simultaneously involved in disease, and being differently affected by the various pathological processes that affect the gland. We should recognize disturbances of both these internal secretions as due to organic disease, as also to *functional* derangement of the parenchyma.

It is probable also that the disturbance of fat absorption in a case of congenital steatorrhea, reported by Garrod and Hurtley,[66] is explainable on the basis of the congenital absence of such an internal secretion. In the same category can probably be included the case of pancreatic infantilism described by Bramwell.[67] Also the occurrence of fatty stools in some cases of exophthalmic goiter, described by Bittorf,[68] in which a general disturbance of polyglandular secretions may exist. The peculiar disease reported by Whipple,[69] in which large deposits of fat and fatty acids occurred in the intestinal and mesenteric lymphatic tissue, may fall into the same category.

Many workers in the field of pancreatic disease have noted a marked improvement in the absorption of both fat and nitrogen on feeding pancreatic extracts or the raw gland (Salomon,[70] Ehrmann,[71] Gross,[54] etc.). This has been held as a proof that the external secretion of the gland controls digestion and absorption. To controvert this argument there are two strong facts. First, with even excessive feeding of commercial extracts of raw gland, no ferments were demonstrable in the intestine, though the absorption of food had markedly improved. Second, my own analyses of the known market products prove them either inactice or so weak as to be incapable of replacing such strong ferments as the gland normally supplies. Yet the improvement in absorption after feeding the gland is beyond question. The explanation lies in the fact that both commercial and raw extracts prob-

63. Hirschfeld: Ztschr. f. klin. Med., 1891, xix, 294.
64. Fitz: Tr. Congr. Am. Phys. and Surg., 1903, vi, 36.
65. Deaver: Jour. Am. Med. Assn., 1911, lvi, 1079.
66. Garrod and Hurtley: Quart. Jour. Med., 1912, vi, 242.
67. Bramwell: Quoted from Robson and Cammidge, The Pancreas, Its Surgery and Pathology, 1907.
68. Bittorf: Deutsch. med. Wchnschr., 1912, xxxviii, 1034.
69. Whipple: Bull. Johns Hopkins Hosp., 1907, xviii, 382.
70. Salomon: Berl. klin. Wchnschr., 1902, xxxix, 45.
71. Ehrmann: Ztschr. f. klin. Med., 1909-10, lxix, 319.

ably contain the internal secretion of the gland in an active form, just as does commercial thyroid or pituitary extract.

We may summarize by saying that cases with distinct increase of fat and nitrogen excretion, not due to intestinal disease or abdominal tuberculosis, and greater than the fat loss due to simple biliary obstruction, are strongly indicative of pancreatic disease. From the absorption test, the diagnosis of the nature of the disease is not vouchsafed, but an impression is gained of the functional activity of the surviving glandular tissue. The absorption test also offers no knowledge of the condition of patency or non-patency of the ducts, nor of the activity of the external secretion.

GENERAL CONCLUSIONS

1. The quantitative examination of duodenal ferments is the most rational and accurate method of studying the external secretion of the pancreas. Diminution of such enzyme activity of the pancreas is a reliable sign of organic disease of the gland. Occasionally, though rarely, a diminution of ferments occurs as a symptom of advanced organic disease elsewhere in the body. Roughly, the diminution of ferments is directly proportional to the extent of organic destruction which has taken place.

2. The absorption of fat and nitrogen from the intestine is independent of the condition of the external secretion, or even of its presence. Absorption may be poor with an intact gland, or good with a gland of which only a fragment survives the disease. The functional activity of the gland, not its organic condition, determines the degree of absorption; this is probably controlled by an internal secretion or hormone.

3. Duodenal ferment tests give the index of the organic condition of the gland. Absorption tests give the index of the functional activity of the pancreas.

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A DIFFERENTIAL STUDY OF COCCIDIOIDAL GRANULOMA AND BLASTOMYCOSIS

I. PATHOLOGY AND BACTERIOLOGY
II. REPORT OF TWO ADDITIONAL CASES OF COCCIDIOIDAL DISEASE *

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To California physicians who have seen a good deal of a disease peculiar to the San Joaquin Valley and described as coccidioidal granuloma there seems to be a confusion on the part of some writers as to its relation to blastomycosis, and indeed, the two have been frequently referred to as one and the same disease. It is our purpose to show how real the confusion is in the literature, and by describing the morphology of the two organisms, their development and appearance in tissue, as well as the reactions found, and finally by a comparison of the effect of each form on inoculated animals, to show that they are distinctly different and not merely different forms of the same organism with different methods of development and different toxicity under varying conditions. This latter view is held by Wright, who in his article on the subject in Osler's "System of Medicine," regards all the cases reported under the names coccidioidal granuloma and blastomycosis as different manifestations of the same disease, which he calls oidiomycosis. He states that the prognosis is bad where the condition is due to infection with sporulating forms, whereas only four of thirty patients died when infection was with budding forms. He discusses briefly Wolbach's morphological studies and animal work with the sporulating forms, and considers the budding forms another phase of the same organism.

Whitman, who studied a case of "systemic blastomycosis" in Denver, recognizes the confusion which exists and in an article on the "Botany of the Organism of Blastomycosis," states that "the organisms in these cases are probably not identical." The importance of budding referred to by many authors as a different morphological characteristic is set aside by him as having no biological significance and no longer

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used by mycologists on account of its being found so widely distributed among fungi. "There is apparently ample ground for the view that the term 'blastomycosis' really includes a wide variety of organisms. Cultures from different cases present considerable variations in morphology and cultural characteristics." An abundance of budding forms marked some, whereas an absence characterized others. Some recovered rapidly under potassium iodid, whereas others progressed obstinately in spite of it.

In his case, Whitman says: "I have observed for the first time the production of mycelia within the tissues." He traces the development of asci in the tissues and states that budding forms are exceedingly rare. He concludes that the plant may belong to the *Monascaceae* or *Exoascaceae* in the *ascomycetes* group, and that a study of the behavior of this organism under conditions natural to the life of related groups would possibly give complete information regarding its botanical position.

Brewer's case of an abscess in the back, connected with the lumbar vertebrae, was studied by Wood, who reported the pus showing large numbers of spherical cells of different sizes (from 10 to 25 microns), each surrounded by a clear gelatinous envelope. No budding was found in the organisms as seen in fresh pus and mycelia were not observed in cultures, which grew easily but slowly on ordinary mediums.

Zinsser discussed the morphological characteristics of the organism found in Brewer's case of blastomycosis. Unlike most forms described, his organism grew poorly on all fluid mediums. Reproduction was by budding, and in no case even in agar and gelatin hanging drops were mycelia observed. In old cultures daughter cells grew and budded again without detachment entirely from the mother cell.

Ewing, in the discussion, reported a case in Coley's service from which an organism with abundant mycelia was cultivated. The organism grew at room temperature and even in the ice-box.

Irons and Graham's case of generalized blastomycosis showed no special points of interest except the belief that the primary lesion was in the lungs. Although they state, as is generally believed, that dissemination is by the blood-stream, two blood-cultures were sterile. Cultures on ordinary mediums showed elevated, mold-like, definitely margined growths with aerial hyphae and mycelia. Budding forms were found in young cultures.

Morris of New York reported as systemic blastomycosis the case of a physician from Texas who infected his finger the year before while dissecting in Chicago. MacNeal and Hjelm, who made the cultures and discussed the diagnosis in the same journal, described the organism

as growing after forty-eight hours' incubation in "radiating mycelial threads." The fresh pus contained double contoured spheres (from 4 to 25 microns). No budding forms or mycelial threads were recognized in the pus, but in growths the mycelia were abundant. They regarded the organism as "identical with that discovered by Posada and Wernicke and first described in the United States by Rixford, who bestowed on it the name *Coccidioides immitis*.

Buschke[1] discussed at length the *"Hefeähnliche Microorganism."* In the oidiomycetic infections of tissue he states that the oval bodies alone are found, mycelia having been observed by no authors. Gilchrist's disease he calls *Oidiomycosis americana*, and the coccidioidal granuloma cases of Ophüls and Moffitt and other American authors he regards as the same affection. He would group them under the heading of blastomycosis.

A detailed review of the literature on coccidioidal disease and blastomycosis has not been attempted by us. Ricketts presented an exhaustive study, in 1901, of the clinical and laboratory aspects of the disease and concluded that the so-called "protozoic disease" (coccidioides) of Posadas, Wernicke and Ophüls, and Gilchrist's blastomycetic dermatitis were various manifestations of the same disease. He described the lesions of blastomycosis consisting of subcutaneous abscesses or granulomatous nodular growths with usually the appearance of a primary papule. The abscesses had a cauliflower-like surface with elevated borders and surrounded by a red, indurated zone. The centers of the lesions showed granulations. Marked epithelial hyperplasia was noted as well as epidermal and subepidermal abscesses. Dense infiltrations of cells, including giant-cells, were noted. Vast numbers of organisms were found in some cases in the pus and granulation tissue. In the viscera small nodular masses and abscesses with and without cheesy material developed. A comparative study of the organisms, including blastomycetes or oidiomycetes and the organism of Ophüls and Moffitt's "so-called protozoic disease," revealed practically no differences. At least there appeared to be no greater differences than between the various strains of blastomycetes. Most of the organisms developed on artificial mediums in one or two days, certain others requiring from two to seven days. He divided the entire group (including Busse's *Saccharomycosis hominis*, and Curtis' *Saccharomycosis humaine*) into three classes, depending on the appearance of growth on culture mediums: (1) elevated, moist, white colonies coalescing to form a fleshy growth of paste-like consistency; (2) granular surface, slightly elevated and on coalescing, more elevated and

1. Buschke: In Kolle and Wassermann's Handbuch der path. Mikroorganismen, xv, 155-210.

eventually a surface looking like a piece of crumpled cloth or mass of earthworms; (3) aerial hyphae and surface white, dry and flour-like and eventually covering sides of container. Some of the organisms produced acetic acid; some fermented glucose and maltose, and others fermented saccharose and lactose, while others had no fermentative activity. One produced indol. A complete study of the "protozoic" organism appears not to have been made. Experimental inoculations showed considerable resistance to infection by the various strains of blastomycetes on the part of guinea-pigs, dogs and rabbits.

In 1904 Wolbach studied a strain of coccidioidal organisms isolated from a patient with subcutaneous lesions. Growth appeared in culture mediums in two to seven days, and after some time it showed on solid mediums an opaque white, elevated center due to aerial hyphae bearing gonidia. In broth globular, white, thistle-down-like masses developed and settled to the bottom of the tube. Saccharose, dextrose and mannite were not fermented. Microscopically, the growth showed radiating masses of coarse, branching, segmented mycelia with distinct membrane. In very old cultures spherical bodies were found resembling those found in tissues. The organism was highly virulent for guinea-pigs and rabbits, and the lesions simulated tuberculosis. Endogenous reproduction was found in the organisms recovered from the animals. No evidences of mycelia or budding processes were noted, although the latter were in some instances simulated owing to juxtaposition of the cells. The development of mycelia from the endosporulating cell as found in pus was studied on agar slides and hanging-drop preparations and the reverse development by intravenously inoculating rabbits and examining sections of organs from twenty-four hours up to several weeks, as well as by the introduction of cultures sealed in collodion capsules into the peritoneal cavity of rabbits. Wolbach stated that "the organism cannot be a blastomyces, neither can it be included in the oidium-like group of Ricketts, since the organisms of that group may reproduce by budding."

In 1907, Hektoen published a detailed review of cases and concluded that they were different diseases. In 1908, Montgomery and Ormsby summarized the literature, and, in 1909, Harter published a book on blastomycosis.

In the ARCHIVES OF INTERNAL MEDICINE, April 15, 1914, a series of eleven case-reports on systemic blastomycosis appeared, together with a pathological, bacteriological and clinical study of the disease by Stober. He obtained cultures from the moldy wood in the homes of the patients and noted a similarity with the cultures from human tissues. Vaccination with blastomycetes was tried in two systemic cases and one cutaneous case; of these one of the former patients

recovered. The vaccine consisted of the filtrate and suspension of the triturated membranes of old bouillon cultures which had been heated at 110 C. Stober inclines to the theory that systemic blastomycosis is primarily a respiratory infection. Blood cultures, in a case reported by Krost, Stober and Moes were positive. (Buschke had previously reported one such case.) The urine also showed blastomycetes, the source of which, as shown at necropsy, was the prostate. This series of articles consists of reproductions from the Cook County Hospital reports, 1910, as noted on page 509 of THE ARCHIVES.

The last summary of recently reported cases of coccidioidal granuloma together with experimental work has been published by MacNeal and Taylor. Anaerobic cultivation of the *Coccidioides immitis* in sterile ascitic fluid and gelatinized horse-serum with pieces of sterile tissue showed on inoculation of pus the development of round-cells and no filaments at the bottoms of the tubes. Such cultures have been kept alive for three weeks. Their work supports the observation of Wolbach that the double-contoured spheres in the animal tissues develop by a direct transformation of septate mycelial threads and not from the chlamydospores. MacNeal and Taylor believe that the disease produced by this organism is an entity and that it is differentiable from blastomycosis, oidiomycosis, etc., and that one point of practical therapeutic differentiation is the value of iodids in blastomycosis and their failure to benefit cases of coccidioidal disease, as emphasized earlier by one of us (Brown). MacNeal and Taylor conclude that coccidioidal granuloma is a definite specific infectious disease and that "*coccidioides immitis* (class *Ascomycetes*) is distinctly different from the budding fungi found in human diseases variously called blastomycosis, oidiomycosis," etc.

The association of coccidioidal granuloma and blastomycosis, as one disease, has been asserted by Wright, Ricketts, Buschke, etc., and a clinical confusion specifically illustrated by Morris' case of reported blastomycosis which was shown on laboratory examination to be coccidioidal disease. Welch believes that they are two differentiable diseases, and Ophüls, Hektoen, Wolbach, MacNeal and Taylor, and others, are of the opinion, as the result of clinical and laboratory observations, that they are separate conditions.

PERSONAL OBSERVATIONS

CASE 1.—H. D. V., aged 24, San Joaquin Valley, California, family and previous history negative, was in the Southern Pacific Hospital from Aug. 16 to 20, 1912, for laryngitis, which was suspected of being tuberculous, although reactions were negative. Readmitted with typhoidal symptoms. Widal positive 1:40 and blood-culture negative. Died March 2, 1913, having been in hospital about two months. Gross examination of organs revealed apparently a disseminated miliary tuberculosis, while microscopically coccidioidal lesions

were found in the spleen, lungs, liver, kidneys, pancreas and adrenals. The organisms showed endosporulation. There were no cultures.[2]

CASE 2.—Service of Dr. S. J. Gardner. J. P., aged 29, Mexican, laborer in section-gang (railroad), Bakersfield, San Joaquin Valley, Cal., was admitted to hospital July 27, 1913. Family history was negative. Patient denied venereal infection. The present infection apparently began three weeks before admission with a nodule on the dorsum of the right great toe. The day before admission it had been lanced and a quantity of pus escaped. He denied injury.

Examination.—Poorly nourished adult. Temperature was 104 F. and only a slight increase in respiratory and pulse-rates. Head, chest and abdomen negative. Right great toe presented two open wounds which were exuding considerable pus. Throughout the course of the disease the temperature fluctuated between normal and 104 F. The foot was kept for varying periods of time in a salt-water bath, and potassium iodid was administered internally in 20-grain doses thrice daily. There was no improvement and the patient was transferred to the roof that the foot might be exposed to the direct sun rays. For a time there was some decrease in the amount of pus. There was progressive decline, however, and about two weeks before death patient became stuporous, and had by this time become markedly emaciated. There was no apparent joint or other bone lesions and no evidences of pleurisy. No cough. Pus from the toe showed typical coccidioidal organisms with endosporulation. Tissue from margin of lesion showed the same. Blood: August 9, hemoglobin 70 per cent.; red blood-cells, 4,760,000; white blood-cells 16,600. Blood-cultures: August 6 and September 15, sterile. Urine: July 29, negative. Wassermann reactions: blood-serum, August 2, negative, and cerebrospinal fluid, September 10, weakly positive (++). The fluid was moderately turbid and under a pressure of 140 mm. Butyric acid and ammonium sulphate reactions positive. Cells 20 (?) per c.mm.

Necropsy Record.—Markedly emaciated body. Bedsores over sacrum. Peritoneum smooth and dry and no adhesions. Spleen moderately enlarged (240 gm.). There were numerous small, firm, grayish nodules of millet-seed size. Liver slightly enlarged (1,620 gm.), dark brown and flabby. Gall-bladder apparently normal as well as stomach. Intestines were moderately congested. Pancreas apparently normal. Both kidneys moderately enlarged (from 200 to 210 gm.). Several millet-sized, whitish areas in cortex and medulla. Adrenals, ureters, bladder and genitalia apparently normal. In left pleural sac a few rather firm adhesions at apex. In left lung (500 gm.) numerous closely aggregated, grayish, firm nodules throughout both lobes. Right pleural sac normal. Right lung (620 gm.) resembled the left. Heart (300 gm.) had a pale, flabby muscle. Valves apparently normal. Beginning of aortic arch showed few small areas of early sclerosis. Retroperitoneal lymph-nodes moderately enlarged and firm. Brain (1,360 gm.) showed a uniformly moderate thickening of the dura mater. Overlying the occipital dura there was a well defined purulent collection which was directly continuous through a necrosed portion of the occipital bone with a larger mass of pus beneath the scalp. There was early necrosis of the left temporal bone. There was extensive necrosis of the upper half of the sternum and the third, fourth and fifth lumbar vertebrae with pus development, as well as the bones of the right great toe.

Microscopic examination showed coccidioidal lesions (endosporulating organisms) in the lungs, liver, kidneys, occipital bone, dura mater, meninges of cervical and dorsal cord, as well as congestion, edema, emphysema and hemosiderosis of lungs; passive congestion of liver, congestion of adrenals, chronic gastro-enteritis, chronic retroperitoneal lymph-adenitis; hyaline changes in right soleus muscle.

2. Carson and Cummins: A Case of Coccidioidal Granuloma (California Disease), Jour. Am. Med. Assn., 1913, lxi, 191.

Cultures made at necropsy and incubated at 37 C. showed within twenty-four hours growth of coccidioidal organisms.

CASE 3.—Service of Dr. J. H. O'Connor. A. R., aged 27, blacksmith helper, Kerto (since August, 1913), San Joaquin Valley, Cal., was admitted to Hospital March 5, 1914. Family and previous personal history negative. Patient denied venereal infection. The present condition apparently began ten days previously with lumbar pains. Patient *stated that he lost about 13 pounds in fifteen days.* He denied injury.

Examination.—Fairly well-nourished man. On admission temperature, pulse and respiration normal. No tenderness in lumbar region. Blood-pressure, systolic, 120 mm. Urine, negative. Two days after admission there was definite localization of tenderness, and a mass noted in the lumbar muscles over the left kidney. On opening there exuded considerable pus in which double-walled endosporulating cells were found. Six weeks after admission, tenderness and swelling of the right wrist and left elbow appeared. At that time there was a moderate purulent discharge from the lumbar lesion, whose walls were of a gray, slimy, necrotic appearance. The Roentgen picture showed no apparent connections with the spine. Throughout the period of illness an irregular temperature was maintained; maximum was 103.4 F. Daily applications for five minutes of gauze saturated with pure phenol were made over a period of seven weeks. Then for four weeks daily applications to the lesion of gauze saturated with 0.25 per cent. solution of crystal violet were made. At no time were there evidences of granulations nor, on the other hand, extended necrosis. During the latter period intravenous injections of crystal violet were given, as follows:

May 8, 100 c.c. of 0.25 per cent.; May 11, 100 c.c. of 1 per cent.

May 16, 100 c.c. of 2 per cent.; May 19, 400 c.c. of 4 per cent.

Slight chills following three injections were probably induced by insufficient heating of the solution rather than by any toxicity of the crystal violet. On May 13, 5,200 c.c. of violet-tinged ascitic fluid were withdrawn; on May 21, 2,250 c.c.; on May 28, 1,040 c.c. The swelling of the wrist and elbow decreased and there was no other apparent bone or joint involvement. Death, June 1. Unfortunately permission for necropsy could not be obtained.

EXPERIMENTAL WORK

The coccidioidal cultures used for animal inoculations and bacteriological study were those from Case 2; the blastomycosis culture was obtained from a systemic case at the city and county hospital through the courtesy of Dr. Harold Hill. A limited bacteriological study was carried out on the strain of coccidioides from Case 3. Other cultures of coccidioides were obtained from Dr. Cook of the University of California and from Drs. MacNeal and Taylor of the Post-Graduate Medical School in New York, and form the basis of study which is not yet completed. Rabbits, guinea-pigs and white rats were inoculated with the coccidioides and blastomycetes, and cats with the coccidioides only.

COCCIDIOIDES

Five Rabbits

Rabbit 1.—December 13: Inoculated *intravenously* 2 c.c. salt solution suspension culture containing spores.

December 29: Killed. Coccidiosis liver. No evidence of coccidioidal disease.

Rabbit 2.—December 13: *Intraperitoneally* 5 c.c. salt solution suspension of culture.

December 29: Killed. Coccidiosis of liver. No evidence of coccidioidal disease.

Rabbit 3.—December 13: *Intrapleurally* as Rabbit 1. December 16 and December 22 negative.

January 5: Pea-sized mass near site of inoculation, but not attached to ribs.

January 23: Chestnut-sized mass.

April 4: Killed. Large mulberry-form abscess intrathoracic at site of inoculation. Small abscess extrathoracic. Left ankle-joint enlarged and contained some pus; at right knee small firm tumor. Coccidioidal organisms in pus.

Rabbit 4.—December 13: *Intratesticularly* (right) as 1. Small bead in epididymis.

December 22: Both testicles enlarged and right indurated.

January 5: Sinus from right.

January 23: Both testicles size of small lemons. No discharge from sinus.

February 28: Killed. Purulent orchitis (bilateral); coccidioidal organisms in pus.

Rabbit 5.—December 13: *Inguinally* (right) as Rabbit 1.

December 16 and 22: Negative.

January 5: Split pea-sized nodule in groin.

January 23: Lima-bean-sized nodule at seat of inoculation.

March 18: Killed. Purulent arthritis of left shoulder and right elbow. Pus showed coccidioidal organsms.

Summary.—Intrapleurally, intratesticularly and in inguinal region, positive. Intravenous and intraperitoneal inoculations, negative.

Nine Guinea-Pigs, Male

Pig 1.—September 3.—Inoculated *intraperitoneally* with 2 c.c. salt solution suspension of pus from Case 2.

September 22: Both testicles somewhat enlarged.

October 2: Sinus from each testicle.

October 21: Killed. Coccidioidal lesions of testicles and spleen.

Pig 2.—September 3: Inoculated as above.

October 27: Indurated ulcer of scrotum.

October 29: Also enlarged right inguinal nodes.

December 4: Testicle healed; inguinal enlargement persisted.

December 16: Died. Coccidioidal involvement of inguinal nodes only.

Pig 3.—September 3: Inoculated as above.

October 27: Both testicles somewhat enlarged.

October 7: Small indurated ulcer of scrotum.

October 29: Also enlarged inguinal nodes, both sides.

December 4: Killed. Large mass in left inguinal region. Some enlargement of axillary groups in both sides. Coccidioidal disease present.

Pig 4.—November 24: Inoculated with 4 c.c. salt solution suspension 4-months'-old culture *intraperitoneally*. December 4. Some induration of both testicles.

December 12. Also slight inguinal enlargement.

December 16: Died. Coccidioidal lesions in left inguinal node and testicle.

Summary.—Intraperitoneal inoculations positive.

Pigs 5, 6, 7 and 8.—December 18: Each inoculated *intraperitoneally* with 1 c.c. salt solution suspension of pus from testicle of Pig 4.

December 21-22: All died. Congestion of lungs, liver, kidneys and adrenals. Cultures from peritoneal fluid sterile.

Pig 9.—December 21: Inoculated *intraperitoneally* 1 c.c. salt solution suspension of peritoneal exudate from one "December 18 pig."

December 25: Died. Congestion heart, kidneys, lungs and liver. Cultures from peritoneal fluid sterile.

Thirteen White Rats

Rat 1.—September 6: Inoculated *subcutaneously* in back with 1 c.c. salt solution suspension pus from Case 2.

March 22: Ulcer at site of inoculation.

October 2: No pus — more induration.

October 19: Died. Negative.

Rats 2 and 3.—September 6: Inoculated *intraperitoneally* as above.

September 14: Some redness and swelling at sight.

October 2: Some induration of testicles.

November 12: Negative.

January 20: Killed. Negative.

Rat 4.—November 24: Inoculated *intraperitoneally* with salt solution suspension of 4-months-old culture.

December 4: Small nodule at site of inoculation.

January 5: Negative.

April 4: Killed. Small firm cysts in liver. Coccidioides in smears and cultures.

Rats 5, 6 and 7.—March 5: Inoculated *intraperitoneally* with 1 c.c. salt solution suspension of pus from testicle of Rabbit 4. Killed March 17 and one only showed coccidioidal organisms in fluid from small hepatic cyst.

Rats 8, 9 and 10.—March 5: Inoculated as above.

April 17: Killed. All negative.

Rats 11, 12 and 13.—March 5: Inoculated *testicularly* as above.

April 17: Killed. All negative.

Summary.—Two of thirteen rats, positive.

Five Cats

Cat 1.—Full grown. September 5: Inoculated *intraperitoneally* with 2 c.c. salt solution suspension pus from Case 2.

September 14: Some swelling at site of inoculation.

September 18-27, October 2: Idem.

October 15: One area size of quarter dollar and several smaller areas.

October 22: Idem.

November 15: One large area of induration.

December 4: Small nodule.

December 12: Idem.

January 5: Negative.

January 28: Killed. All organs grossly negative. Microscopically, few coccidioidal organisms found in small dense cellular collections in lungs.

Cat 2.—Full grown. September 5: Inoculated *subcutaneously* in back as above.

September 14: Some swelling at site of inoculation.

September 18 and 27 and October 2: Idem.

October 7: Small area of induration.

October 22: Area of fluctuation.

October 29: Ulcer. Coccidioidal organisms in smears.

November 5: Ulcer healing.

December 4: Ulcer healed.

December 12: Negative.

January 28: Killed. Few small whitish nodules in liver. Smears negative. Other organs including brain and cord grossly negative. Microscopically few coccidioidal organisms in lungs as above.

Cat 3.—Kitten. September 14: Inoculated *intraperitoneally* with 2 c.c., as above.

January 28: Killed. All organs grossly and microscopically negative.

Cat 4.—Kitten. September 18: Inoculated *intraperitoneally* as above.
September 22: Negative.
September 27 and October 2: Two pea-sized masses at site of inoculation.
October 7 and 22: Moderate increase in size.
November 15: Negative.
January 28: Killed. All organs negative grossly and microscopically.

Cat 5.—Kitten. September 18: Inoculated *subcutaneously* in back. At above dates of examination negative.
January 28: Killed. All organs negative grossly and microscopically.

Summary.—Full-grown cats, positive. Kittens, negative.

BLASTOMYCETES

Four Rabbits

Rabbit 6.—December 13: Inoculated *intraperitoneally* with 5 c.c. salt solution suspension of 5-months-old culture.
December 16 and 22: Negative.
January 5: Abdomen somewhat boggy.
January 23: Killed. No evidence of blastomycosis.

Rabbit 7.—December 13: Inoculated *inguinally* as above.
December 16: Negative.
December 22: Died of pneumonia. No evidence of blastomycosis.

Rabbit 8.—December 13. Inoculated *intrapleurally* with 5 c.c. as above.
December 22 and 23, January 5 and 23, April 16: Negative.
April 17: Killed. No gross evidence of blastomycosis.

Rabbit 9.—December 13: *Intratesticularly* (right) with 0.75 c.c. as above.
December 16: Some swelling of the testicle.
January 5: Testicle as large as a lime.
April 16: Apparently normal.
April 17: Killed. Tunica vaginalis testis contained two split-pea-sized nodules containing thick pus in which blastomycetes (budding forms) were found. Right lower eyelid was covered with thick pus and small papillary projections were noted when pus was removed. Blastomycetes were found in pus.

Summary.—Intratesticular inoculation positive. Intrapleural, inguinal and intraperitoneal inoculations negative.

Three Guinea-Pigs, Male.

Pig 10.—November 24: Inoculated *intraperitoneally* with 4 c.c. salt solution suspension of 4-months culture.
December 22: Boggy abdomen.
Dec. 26: Died. Congestion of liver and kidneys. Abscess in right epididymis containing drop of pus. Blastomycetes in smears and cultures.

Pig 11.—December 27: Inoculated *intraperitoneally* with 2 c.c. salt solution suspension of pus from epididymis of Guinea-Pig 10.
January 13: Died. Congestion of lungs and liver; acute nephritis; acute follicular splenitis. No blastomycetes.

Pig 12.—Inoculated as Pig 11.
April 17: Killed. No evidence of blastomycosis.

Summary.—Two of three intraperitoneal inoculations negative.

White Rats

Rat 14.—Inoculated *intraperitoneally* with 2 c.c. salt solution suspension of 4-months-old culture.
December 4: Small nodule at site of inoculation.
April 4: Killed; negative.

GENERAL SUMMARY OF ANIMAL INOCULATIONS

White rats seem to be markedly insusceptible to infection with coccidioides and blastomycetes; cats to coccidioides. Rabbits and guinea-pigs appear to be more susceptible to coccidioidal than to blastomycetic infection. No lesions were found in the cerebrospinal tissues. Passage through animals would seem to exalt the virulence of coccidioides as shown by five guinea-pigs (5, 6, 7, 8 and 9).

CULTURAL STUDIES

Both coccidioides and blastomycetes grew well on agar of strongly acid and alkaline reactions. Dextrose, dextrin, lactose, saccharose, maltose, raffinose, mannite, galactose, esculin, sorbit, agaricin, rhamnose, adonite, xylose, arabinose, mannose and salacin were not fermented by the coccidioides or blastomycetes. The addition of potassium iodid to mediums for facilitating the growth of the coccidioides or blastomycetes by inhibiting the growth of contaminating bacteria, was not employed in the isolation of the organisms from pus, as it was found that after a short time they overgrow the bacteria.

It was noted that the blastomycetes tubes incubated at 37 C. did not grow so well or so rapidly as when they were kept at room temperature. This fact has been called attention to by Ophüls, whose further statement that coccidioides grows best at 37 C. we also confirm.

Of fifty dyes in neutral agar the violets uniformly showed the greatest inhibitive action on the coccidioides, blastomycetes and staphylococci, and the greens ranked second. The blue dyes varied considerably from the strongest, methylene blue BX and brilliant cresyl blue to the weakest, trypan blue and china blue. Such dyes as safranin, congo red and victoria blue showed well marked differences in their action on the respective organisms. A relatively marked inhibition was effected by safranin on staphylococci. The reverse was true of congo red. Victoria blue exerted a well marked inhibition of the growth of blastomycetes and no inhibition of the coccidioides and staphylococci. Purpurin, orange G, rubin S, sudan III, methyl red, etc., were quite inert. (Some poorly soluble.) Uniformly, the most inhibitive dye was

TABLE 1.—Reactions of Staphylococci, Blastomycetes and Coccidioides with Various Dyes of Different Strengths.*

		0.0005	0.001	0.0025	0.005	0.01	0.02	0.05	0.1	0.25	0.4	0.5	0.6	0.75	0.9	1.0	1.25	1.5	1.75	2 c.c.
VIOLET DYES		0.01%	0.02%	0.05%	0.1%	0.2%	0.4%	0.1%	2%	5%	8%	10%	12%	15%	18%	20%	25%	30%	35%	40%
Cresyl-Echt-Violet	B	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—
	C	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—
Crystal Violet	B	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Dahlia	B	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Gentian Violet	B	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Hoffmann's Violet	B	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Methyl Violet 1 B.	B	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Methyl Violet 2 B.	B	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Methyl Violet 5 B.	B	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Methyl Volet 6 B.	B	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Methyl Violet Bx.	B	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Rosanilin Violet	B	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
GREENS																				
Brilliant Green	B	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—

* Explanation of abbreviations in table: B, blastomyces; C, coccidioides; S, staphylococcus (from a case of osteomyelitis). 5 c.c. neutral agar per tube. Saturated aqueous solution of dyes from which serial percentages (0.01 to 40) were made. Tubes autoclaved for twenty minutes.

TABLE 1.—(*Continued*)

		0.0005	0.001	0.0025	0.005	0.01	0.02	0.05	0.1	0.25	0.4	0.5	0.6	0.75	0.9	1.0	1.25	1.5	1.75	2 c.c.
		0.01%	0.02%	0.05%	0.1%	0.2%	0.4%	0.1%	2%	5%	8%	10%	12%	15%	18%	20%	25%	30%	35%	40%
Emerald Green	B	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Iodin Green	B	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Janus Green	B	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
Malachite Green	B	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
Methyl Green	B	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—
BLUES																				
Anilin Blue	B	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Brilliant-Cresyl-Blue	B	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
China Blue	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl Blue	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methylene-Blue Bx	B	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Methylene-Blue, med. pur.	B	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
Methylene-Blue, rectif.	B	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—
	C	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
	S	+	+	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—

		0.0005	0.001	0.0025	0.005	0.01	0.02	0.05	0.1	0.25	0.4	0.5	0.6	0.75	0.9	1.0	1.25	1.5	1.75	2 c.c.
		0.01%	0.02%	0.05%	0.1%	0.2%	0.4%	0.1%	2%	5%	8%	10%	12%	15%	18%	20%	25%	30%	35%	40%
Nile Blue Sulphate	B	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	C	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	S	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Toluidin Blue	B	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
	C	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
	S	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Trypan Blue	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Victoria Blue	B	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REDS																				
Carmin Rubr. Opt.	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Congo Red	B	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	C	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Corallin Water, Sol.	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Eosin Watery Blue	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Erythrosin	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−
Fuchsin Basic	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematoxylin	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Magdala Red	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
Methyl Red	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* Explanation of abbreviations in table: B, blastomyces; C, coccidioides; S, staphylococcus (from a case of osteomyelitis). 5 c.c. neutral agar per tube. Saturated aqueous solution of dyes from which serial percentages (0.01 to 40) were made. Tubes autoclaved for twenty minutes.

TABLE 1.—(*Continued*)

		0.0005	0.001	0.0025	0.005	0.01	0.02	0.05	0.1	0.25	0.4	0.5	0.6	0.75	0.9	1.0	1.25	1.5	1.75	2 c.c.
		0.01%	0.02%	0.05%	0.1%	0.2%	0.4%	0.1%	2%	5%	8%	10%	12%	15%	18%	20%	25%	30%	35%	40%
Ponceau P. R.	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pyronin	B	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
	C	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
	S	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Purpurin	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rosanilin Base	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rosanilin Hydrochlorid.	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
Rubin S.	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Safranin	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–
	S	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Sudan III	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trypan Red	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bismarck Brown	B	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
	C	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–
Fluorchrome	B	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
	C	+	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–
	S	+	+	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Fluorescein	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Orange G.	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

crystal violet, but for the blastomycetes methyl violet 1 B and 6 B. Crystal violet and Hofmann violet were of equally high potentiality for the coccidioides and blastomycetes. Intraperitoneal inoculations of aqueous solutions of crystal violet in 0.25, 0.5, 1 and 2 per cent. concentration into guinea-pigs and white rats produced no toxic effects. As before noted, this dye was made the basis of therapy in Case 3. Safranin agar in 10 to 20 per cent. strength may prove of value in the isolation of coccidioides from pus and in 10 to 15 per cent. strength, in the isolation of blastomycetes.

The primary growths of the coccidioides from Cases 2 and 3 required two to ten days for their appearance, except in the former, in which a few tubes of slant agar were thickly streaked with pus, and growth appeared within twenty-four hours. There is a tendency primarily for the pyogenic organisms to outstrip the coccidioidal growth, but in the course of two weeks the former growth is practically obliterated. After several subculturings of Strain 2, growth appeared in twenty-four hours. This strain showed the initial characteristic of fine, white threads radiating out over the agar surface, while that from Case 3 showed a whitish "cottony" surface without such radiations. As the agar slants became covered with growth, both strains assumed a somewhat dry, wrinkled, fleshy, brownish-white appearance. Especially in Strain 2 there was early penetration of the agar, and in the thinner drier portions of the medium (top of slant) there was an early development of the whitish "cottony" growth. This appearance was not general until the cultures became old and dry. Strain 3 has not been studied sufficiently long to determine this point. In both strains the growth after a time was of irregular thickness, being in places somewhat elevated and densely adherent to the medium, so that it was necessary for subinoculation to remove small pieces of agar. Strain 2 on the "dye-agars" developed in many tubes well-marked aerial hyphae as a primary growth. With some dyes, notably eosin, orange G and the rosanilins, general growth was more rapid and luxuriant than on the plain agar. Those cultures developed early the whitish, dry, "cottony" growth in dense masses projecting from the surface of the medium. All tubes were incubated at 37 C.

Early in the period of subculturing our strain of blastomyces, seven to ten days elapsed before there were evidences of growth, but later in many tubes this period was reduced to two or three days. The primary growth in all plain agar slants consisted of round, moist, flat colonies composed of radiating whitish lines. Later coalescence took place and the surfaces dried and cultures assumed the appearance as described by Ricketts "(3) aerial hyphae and surface white, dry and flour-like and eventually covering sides of tube." For subinoculation

this growth could easily be removed without puncturing the agar. The eosin, orange G and rosanilin agars favored luxuriant growths. On practically all of the dye-agars primary growth was manifested by aerial hyphae. All tubes were kept at room temperature.

Microscopically, both the coccidioides and blastomycetes in animal tissues presented the characteristic endosporulation and budding processes, respectively. Neither in the pus nor in the solid tissues at any time could we demonstrate budding forms in coccidioidal disease, nor evidences of ensporulation in blastomycosis. No mycelia were found in the tissues in either disease. In cultures of both types of organisms, the already well-described cylindrical, septate, branching filaments with later spore development were noted. Agar slides, as described by Wolbach, were use to study the development of filaments from the cells as found in pus. We found the most satisfactory stain of the culture material to be eosin and methylene blue, but in pus the cells were readily studied on adding a dilute (4 per cent.) caustic soda solution.

The initial growth of coccidioides on culture mediums is rapid, for, if a quantity of pus is inoculated, growth in some tubes has been noted within twenty-four hours. Blastomycytes require in their initial growth about ten days to two weeks for evidence of definite development, but after prolonged cultivation growth is seen in four to seven days. The optimum temperature for coccidioides is 37 C., and for blastomycetes about 20 C. Some strains of blastomycetes eventually show luxuriant growth to the extent that the sides of the container are invaded, whereas the growth of coccidioides seems always to be confined to the mediums.

Animal inoculation yielded well-defined endosporulating organisms of coccidioidal disease, and in the pus of blastomycosis only the budding forms.

SUMMARY

There are well-defined differences in the pathogenicity of the two diseases, coccidioidal granuloma being always fatal, and often rapidly fatal in man, while blastomycosis is commonly not so, except for the systemic cases, in which the organisms were found associated with bacteria of known and unknown pathogenicity (Zinsser, Hektoen, Gilchrist and Stokes). The clinical and pathologic aspects of coccidioidal disease are those more closely resembling tuberculosis, as there is a greater predilection for the lymphatic system than there is in blastomycosis, and cutaneous lesions are likely to be more ulcerative. There appears but one reported case of coccidioidal disease in the female sex, whereas there have been many of blastomycosis. Iodids have temporarily benefited many, and apparently cured a few, blasto-

mycosis patients, whereas they have had no effect on the rapidly progressive lesions and toxemia of coccidioidal granuloma.

In the reported cases of coccidioidal granuloma and blastomycosis confusion is common as to clinical, pathological and bacteriological characteristics. The two organisms appear closely related, having much in common, but with such differences as to justify the conclusion that they are distinct entities. Morphologically, in pus and solid tissues they are differentiable by the endosporulation in the one and budding in the other. Cultural differences are not so pronounced, although we have found that growth is initially more rapid with coccidioides. Blastomycetes appear to grow best at room temperature (20 C.); coccicioides at 37 C. The table of dyes shows some differences in the inhibition of growth. Rabbits and guinea-pigs are more resistant to blastomycetic than to coccidioidal infection.

These appear to be sufficient reasons to justify the consideration of coccidioidal disease and blastomycosis as two different diseases. A second paper will deal with the diseases from serological, vaccine and chemotherapeutic points of view.

BIBLIOGRAPHY

Coccidioidal Granuloma.

Brown, P. K.: Coccidioidal Granuloma, Report of Seventeenth and Eighteenth Cases, California State Jour. Med., December, 1906.

Brown, P. K.: Coccidioidal Granuloma, Jour. Am. Med. Assn., 1907, xlviii, 743; A Fatal Case of Coccidioidal Granuloma, 1913, lxi, 770.

Buschke, A.: Die Sprosspilze; Handbuch der path. Mikroorganismen, v, 155-210.

Carson and Cummins: A Case of Coccidioidal Granuloma (California Disease), Jour. Am. Med. Assn., 1913, lxi, 191.

Gardner and Halton: California State Jour. Med., 1904, ii, 386.

Hektoen: Systemic Blastomycosis and Coccidioidal Granuloma, Jour. Am Med. Assn., 1907, xlix, 1071.

MacNeal and Hjelm: Note on a Mold, Coccidioides, Found in a Case of Generalized Infection in Man, Jour. Am. Med. Assn., 1913, lxi, 2044.

MacNeal and Taylor: Coccidioides Immitis and Coccidioidal Granuloma, Jour. Med. Research, 1914, xxx, No. 3, p. 261.

Montgomery: Brit. Jour. Dermat., 1900, xii, 343.

Montgomery, Ryfkogel and Morrow: Jour. Cutan. Dis., 1903, xxi.

Ophüls: Further Observations on a Pathogenic Mold Formerly Described as a Protozoan, Jour. Exper. Med., 1905, vi, 443.

Wolbach: The Life Cycle of the Organism of Dermatitis Coccidioides, Jour. Med. Research, 1904, xiii, 53.

Wright: Osler's System Med., i, 346.

Zinsser: Proc. New York Path. Soc., 1907, vii.

BLASTOMYCOSIS

Austin: Cutaneous Blastomycosis, Jour. Ind. State Med. Assn., Oct. 15, 1911.

Bassoe: Jour. Infect. Dis., 1906, iii, 91.

Bechtel, R. E., and LeCount, E. R.: Case of Systemic Blastomycosis with Necropsy, THE ARCHIVES INT. MED., 1914, xiii, 609.

Bevan, Arthur Dean: Treatment of Actinomycosis and Blastomycosis with Copper Salts, Jour. Am. Med. Assn., 1905, xlv, 1492.

Boughton, T. H., and Clark, S. N.: A Case of Blastomycosis, THE ARCHIVES INT. MED., 1914, xiii, 594.

Boughton, T. H., and Stober, A. M.: A Case of Systemic Blastomycosis with Recovery, THE ARCHIVES INT. MED., 1914, xiii, 599.

Buschke: Virchows Arch. f. path. Anat., 1896, cxliv, 360; and Kolle and Wassermann's Handbuch der Path. Mikroorganismen, v, 155.

Busse: Centralbl. f. Bakteriol., 1896, xli, 175; Virchows Arch. f. path. Anat., 1895, cxl, 23.

Christensen, C., and Hektoen, L.: Two Cases of Generalized Blastomycosis, Jour. Am. Med. Assn., 1906, xlvii, 247.

Churchill and Stober: THE ARCHIVES INT. MED., 1914, xiii, 568.

Cleary: Tr. Chicago Path. Soc., 1904, iv, 105.

Coley and Tracy: Jour. Med. Research, 1907.

Curtis: Ann. de l'Inst. Pasteur., 1896, x, 449.

Davis: Immunologic Reactions of Oidiomycosis in the Guinea-Pig, Jour. Infect. Dis., March, 1911.

De Beurmann and Gougerot: Tribune méd., 1909, xlii, 501, 517; Les Nouvelles Mycosis, Paris, 1912.

Eisenstaedt, J. S., and Boughton, T. H.: A Case of Systemic Blastomycosis, THE ARCHIVES INT. MED., 1914, xiii, 617.

Fowler: Blastomycosis, a Report of Two Cases, Long Island Med. Jour., 1909, iii, 423.

Frick: Cutaneous Blastomycosis: Report of Three Cases, Jour. Missouri State Med. Assn., January, 1913.

Gilchrist: Brit. Med. Jour., 1902, ii, 1321.

Hamburg: Jour. Infect. Dis., 1907, iv, 201.

Harter, André: De la blastomycose humaine, Nancy, France, 1909.

Herrick, James B.: Generalized Blastomycosis: Report of a Case with Recovery, Jour. Am. Med. Assn., 1907, xlix, 328.

Hill and Dickson: California State Jour. Med., 1914, xii, No. 3.

Irons and Graham: Jour. Infect. Dis., 1906, iii, 666.

Jackson, H.: A Case of Systemic Blastomycosis, THE ARCHIVES INT. MED., 1914, xiii, 607.

Krost, R. A., Stober, A. M., and Moes, M. J.: A Case of Systemic Blastomycosis, THE ARCHIVES INT. MED., 1914, xiii, 557.

LeCount and Meyers: Jour. Infect. Dis., 1907, iv, 187.

Lewison, M., and Jackson, H.: A Case of Systemic Blastomycosis, THE ARCHIVES INT. MED., 1914, xiii, 575.

Longenecker: Jour. Kansas Med. Soc., January, 1912.

Montgomery and Ricketts: Jour. Cutan. and Genito-Urin. Dis., 1901.

Morris: A Case of Systemic Blastomycosis, Jour. Am. Med. Assn., 1913, lxi, 2043.

Myers, H. J., and Stober, A. M.: A Case of Systemic Blastomycosis, THE ARCHIVES INT. MED., 1914, xiii, 585.

Owens, Eisendrath and Ready: Ann. Surg., 1899, xxx.

Posey, Carpenter, Hosmer and Smith: Peculiar Blastomycetoid Organisms Met in Two Cases of Parasitic Conjunctivitis, Univ. Penn. Med. Bull., November, 1908.

Ravogli: Lancet Clinic, May 13, 1911.

Ricketts: Blastomycetic (Oidiomycetic) Dermatitis and Its Organisms, Philadelphia Med. Jour., 1901, vii, 797; also Jour. Med. Research, 1901, vi, 377.

Riley and LeCount: THE ARCHIVES INT. MED., 1914, xiii, 614.

Rusk: A Case of Pulmonary, Cerebral and Meningeal Blastomycosis, Proc. New York Path. Soc., 1910, x, 48.

Rusk and Farnell: Systemic Oidiomycosis: A Study of Two Cases Developing Terminal Oidiomycetic Meningitis, Univ. California Publ. in Path., ii, No. 8, p. 47.

Shaffner, P. F.: A Case of Systemic Blastomycosis, THE ARCHIVES INT. MED., 1914, xiii, 621.

Shepherd: Fatal Case of Blastomycosis, Jour. Cutan. Dis., November, 1911.

Smith: Note upon Additional Cases of Blastomycosis, Univ. Penn. Med. Bull., 1909-10, xxii, 362.

Snoke, J. H., and Strick, E. J.: A Case of Suspected Pulmonary Blastomycosis, Jour. Am. Med. Assn., 1912, lxix, 2056.

Stiel: Blastomycetes in Conjunctivitis Resembling Trachoma, Deutsch. med. Wchnschr., Dec. 12, 1912.

Stober, A. M.: Systemic Blastomycosis: a Report of Its Pathological, Bacteriological and Clinical Features, THE ARCHIVES INT. MED., 1914, xiii, 509.

Sutton: The Occasional Clinical Resemblance of Blastomycosis and Syphilis to Sporotrichosis, Jour. Am. Med. Assn., 1913, lx, 115.

Walker, James W., and Montgomery, Frank Hugh: Further Report of a Previously Recorded Case of Blastomycosis of the Skin, Jour. Am. Med. Assn., 1902, xxxviii, 867.

Washburn, Robert G.: Systemic Blastomycosis, Jour. Am. Med. Assn., 1911, lvi, 1095.

Whitman: A Contribution to the Botany of the Organism of Blastomycosis, Jour Infect. Dis., July, 1913, p. 85.

THE SPECIFIC GRAVITY OF THE HUMAN BODY*

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HISTORICAL

Specific gravity is one of the first fundamental principles of hydrostatics, and its discovery is ascribed to Archimedes (287 B. C.). The incentive to his discovery and the method of his reasoning is described in the following story which carries with it the flavor of the East:

King Hiero ordered his silversmith to make for him a crown of pure gold. When the crown was brought before him he suspected the honesty of the silversmith, and desired to know if Archimedes could devise a way of testing the question without injuring the crown. The philosopher pondered the problem for a long time without succeeding, but one day as he stepped into a bath his attention was attracted by the overflow of water. A new train of ideas was started in his ever receptive brain. Wild with enthusiasm he sprang from the bath, and forgetting his robe, he dashed along the streets of Syracuse shouting "Eureka! Eureka!" The thought that came to his mind was this: that any heavy substance must have a bulk proportionate to its weight, bulk for bulk, and that the way to test the bulk of such an irregular object as a crown was to immerse it in water. The experiment was made. A lump of pure gold of the weight of the crown was immersed in a certain receptacle filled with water and the overflow was noted. Then a lump of silver of the same weight was similarly immersed. Lastly, the crown itself was immersed, and of course — for the story must not lack in dramatic sequel — was found bulkier than its weight of pure gold. The silversmith confessed that he made the crown of an alloy of silver and gold. The historian of science[1] traces the first record of this story to the tenth century, but the principles of specific gravity have fortunately been tersely and lucidly stated by Archimedes himself in his book on floating bodies.[2]

DEFINITION

Specific gravity is the ratio between the weights of equal volumes of any substance and of some other, chosen as a standard. For solids and liquids, distilled water at 62 F. and barometer 30 is taken as a standard.

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1. Cajori: History of Physics, 1899, p. 4.
2. Works of Archimedes, edited by T. L. Heath. Cambridge University Press.

According to the law of Archimedes, a solid floating or immersed in a liquid loses weight equal to that of the volume of liquid it displaces, hence,

$$\frac{\text{Weight of solid}}{\text{Loss of weight}} = \frac{\text{Specific weight of the solid}}{\text{Specific weight of the liquid}}$$

And, since the specific weight of the liquid is unity, hence

$$\text{The specific gravity of the solid} = \frac{\text{Weight of solid}}{\text{Loss of weight}}$$

In other words, the specific gravity of the solid equals its own weight divided by the weight of the volume of liquid it displaced.

THE PIONEERS: OBSERVATIONS ON THE SPECIFIC GRAVITY OF SEPARATE ORGANS OF THE HUMAN BODY

The older anatomists and physiologists paid considerable attention to the study of the specific gravity of the different organs of the human body.[3] John Davy[4] (1828) made careful observations on the specific gravity of some eighty different tissues and organs and he expressed the hope "that the physiologist will receive them as a contribution to his science, and that the pathologist may derive from them a help to the eye and a more certain criterion of organic change than we yet possess. . . . The results are almost demonstrative that organic diseases of the viscera cannot take place without altering their specific gravity; and if it is so, it is not improbable that this precise quality may be made an index of the kind and degree of organic changes."

As far as the available literature at my disposal goes,[5] the credit of the earliest record of observations on the specific gravity of the human body belongs to John Robertson[6] (1814), librarian of the Royal Society. He constructed a cistern 72 inches (183 cm.) long, 30 inches (76 cm.) wide and 30 inches (76 cm.) deep, and having procured ten men for his purpose, the height of each was taken and his weight, and afterward they plunged successively into the cistern. A rule or a scale graduated to inches and decimal parts was fixed to one end of the cistern, and the height of the water shown was noted before each man went in, and to what height it arose when he immersed himself under its surface.

One of the reasons, Mr. Robertson says, that induced him to make these experiments was a desire to know what quantity of timber would

3. See bibliography, Specific Gravity of Separate Parts of the Human Body, at end of article.

4. Davy, John: Trans. Med. Soc., Edinburgh, 1829, iii, 436; also in Researches, Physiological and Anatomical, London, 1839, ii, 253.

5. See bibliography Specific Gravity of the Human Body at end of article.

6. Robertson, John: Philosophical Transactions, 1, Art. 5. Quoted in Hutton's Philosophical and Mathematical Dictionary, London, 1815. (Article, Specific Gravity.)

be sufficient to keep a man afloat in water, thinking that most men were specifically heavier than river or common fresh water; but the contrary appears from trials made, for except the first, every man was lighter than an equal bulk of fresh water and much more so than sea water, so that, "if a person who falls in water had presence of mind enough to avoid the fright usual on such occasions they might be preserved from drowning, and a piece of wood no larger than an oar would buoy a man partly above water as long as he had strength or spirit to keep up his hold."

One hundred years have passed since Robertson made his crude experiments, and yet the number of observers who followed him can be counted on the fingers of one's hands, and the number of observations barely reach the two hundred mark, including the experiments made on cadavers (Herman[7]). Nevertheless these pioneers deserve our gratitude for having established a few definite and valuable facts in connection with this interesting but neglected study, and especially for having discarded the crude contrivance of Robertson's wooden box-cistern in which the material, the shape and the size gave rise to innumerable sources of error, to which we will refer later.

THE AUTHOR'S SPECIFIC GRAVITY APPARATUS

The Volumometer.—The tank is constructed of sheet iron, cylindrical in shape, 64 cm. in diameter and 165 cm. in height. The vessel is reenforced by a steel hoop at about its middle. To avoid injury to the subject while entering and leaving the tank, the sharp upper brim is flanged. The level of the water in the tank is indicated on the water-gage made of Scotch glass, 3 cm. in diameter, graduated in centimeters and millimeters. By means of a rubber tube attached to the water-supply, cold and hot water[8] can be poured into the tank and the temperature tested and regulated either by the tactile sense or by using a water thermometer. The tank is emptied through the outflow pipe attached to the lower end of the water-gage. To dispel the fear of entering a dark reservoir, an electric lamp with a strong reflector illuminates the interior of the tank. After the experiment, if necessary, the tank can be sterilized. To facilitate thorough draining, the tank is placed on a wooden block four inches thick. The entering and the leaving of the tank is facilitated by the use of suitable stepladders. It

7. Herman: Quoted by Buhl in Mitt. a. d. Pathol. Inst. z. München., 1878, p. 4.

8. The quality and temperature of the water have no appreciable influence on its specific gravity. The specific gravity of water at 4 C. is 1.000, and at 40 C. is only 1.00757 (Rossetti, Poggendorff's Ann. d. Physik u. Chemie., 1871, v, 268). The difference between distilled water and any other fresh water is nil.

need not be remarked that during the experiment the tank contains nothing but water and the body whose specific gravity is to be determined.

The Computation of the Content of the Volumometer.—The solid content of a cylinder is computed according to the well-known formula, $\pi r h$ (in which π represents circumference, r radius and h height). The diameter of our tank being 64 cm., the radius is 32 cm. The circumference π is 3.14. Therefore the content of each longitudinal

The volumometer, an apparatus for determining the specific gravity of the human body.

centimeter of the tank ought theoretically to be $3.14 \times 32 \times 32 \times 1 =$ 3,215 c.c. (decimals omitted). On carefully measuring our tank by filling it with water from a graduate and noting the number of cubic centimeters it required to raise the level 1 cm., it was found that each centimeter gave a slightly different reading, but each ten centimeters throughout the whole height of the tank gave the same reading, namely, 3,240 c.c., the difference between the theoretical formula and the actual measurements being only 25 c.c. As the difference is practically a negligible quantity, the value of 3,240 c.c. for each centimeter of the tank was used in my calculations.

WHERE DOES THE GAIN OR LOSS IN BODY WEIGHT GO?

All previous investigators had one end in view, namely, to find by means of the difference in the specific gravity the change in the increase or the diminution of the bulk of the body as compared to the weight. A man weighing, say 176 pounds, when immersed in the tank displaces 79,380 c.c. of water. His specific gravity will be 1.006. Later the man was found to have gained 10 pounds. He has increased in bulk and consequently he should displace a larger volume of water. This is found to be so. He displaces 84,000 c.c. His specific gravity is now 0.990. Or, in other words, when the man weighed 176 pounds he was heavier than water; now that he weighs 10 pounds more he is lighter than water. Since the specific gravity of all tissues except fat[3] is higher than water, if the increase had been due to the enlargement of the muscular or bony tissues his specific gravity would have been the same or higher than before. But the specific gravity has been found lower and therefore it is evident that the increase in his bulk was primarily due to an increase in his fatty constituents. Such a procedure is of diagnostic value.

It was my endeavor, however, to solve an entirely different problem. Let us take the case above referred to of the man having gained 10 pounds; the question arises: How can we locate the gain? In other words: How have the 10 pounds distributed themselves over the body? Have they spread themselves uniformly through the body or not? If the additional bulk has not been distributed uniformly, is there a definite relative increase in the various sections of the body? Have 3 pounds been added to the upper extremity and 7 pounds to the lower extremity, or vice versa? The same question is of course applicable to a man who has lost 4,536 kg. (10 pounds). As far as I could trace, not only has there never been made an attempt to solve this problem, but it has never even been propounded.

In order to arrive at a solution, I reasoned as follows: A human body, being immersed to the knees, will displace a certain volume of water, say 5,000 c.c. The same man immersed to the arch of the pubic bone will displace 15,000 c.c. Immersed to the arch of the ribs, he will displace 50,000 c.c. It is evident that the volume of his feet and legs will be 5,000 c.c., the volume of his thighs will be 10,000 c.c., and the volume of his abdomen from the pubic bone to the arch of the ribs will be 35,000 c.c. After the man has gained 10 pounds he is again immersed in the tank and the same measurements taken. All the readings will show a perceptible change, from which the calculation as to the relative increase in volume can easily be ascertained.

AUTHOR'S METHOD OF TAKING THE VOLUMETRIC MEASUREMENTS OF THE HUMAN BODY

Before the experiment begins the subject should undergo a physical examination. Any deviation from the normal should be noted. The bowels should have moved on that day and the urine be voided before the experiment. I have limited the measurements to the following: (1) the height; (2) the circumference of the chest at the nipple line in normal breathing; (3) the circumference of the abdomen at the umbilical line; (4) the spread of the arms from the tip of the fingers of one hand to the tips of the other hand; (5) the distance from the umbilical line to the floor; (6) the distance from the nipple line to the floor; (7) the distance from the prominence of the thyroid cartilage to the floor.

Immediately preceding the entrance into the tank, the subject should be carefully weighed. The tank is filled to the level of 70 cm. with warm water at a temperature of about 80 F. The subject climbs on the step-ladder and descends into the tank on a small ladder, which hangs on hooks from the rim of the tank. The small ladder is then removed. The level of the water in the tank is indicated on the water-gage. As we know the distance from the umbilicus to the floor, we can readily bring the level of the water to the umbilical line either by adding or letting out a certain quantity of water. The level of the water at the umbilical line is then noted. The small ladder is again introduced into the tank and the subject leaves the tank. The level of the water in the tank is noted. Deducting the second reading from the first gives the number of centimeters which the section of the body from the umbilicus down has raised in the tank. Multiplying this number of centimeters by 3,240 (cubic centimeters to 1 cm. height of tank) gives the number of cubic centimeters which the volume of the body from the umbilical line down displaced. More water is poured into the tank, say to the level of 90 cm. The subject again enters the tank and the level adjusted to the measurements of the nipple line as per measurements made before the experiment began, and the level on the water-gage noted. The subject leaves the tank and the lowered level is again noted. The second reading is subtracted from the first and the difference multiplied by 3,240 and thus the number of cubic centimeters displaced by the volume of the body from nipple line down is obtained. Subtracting from this the number of cubic centimeters obtained from the displacement of the body from umbilical line down we get the amount of water displaced by the volume of the body from the nipple line to the umbilical line. The same is repeated for the section of the body from the prominence of the thyroid cartilage to the nipple line.

TABLE OF AUTHOR'S OBSERVATIONS ON THE SPECIFIC GRAVITY OF THE ENTIRE BODY AND ON THE SPECIFIC EQUIVALENT OF CERTAIN PARTS OF THE BODY

No.	Age	Weight, Kg.	Height, Cm.	Chest, Cm.	Abdomen, Cm.	Spread of Arms, Cm.	Head		Neck to Nipple		Nipple to Umbilicus		Umbilicus to Floor		Entire Body	
							Volume, C.c.	Equivalent, Sp. Gr.	Volume, C.c.	Equivalent, Sp. Gr.	Volume, C.c.	Equivalent, Sp. Gr.	Volume, C.c.	Equivalent, Sp. Gr.	Volume, C.c.	Specific Gravity
1	53	78.926	145	100	103	174	3,888	22.1005	.....	3.6099	.....	.....	.....	.....	79,380	0.994
2	58	78.472	145	100	....	174	3,888	20.1574	21,384	3.7870	14,904	5.2702	38,232	2.0522	78,408	1.000
3	52	78.019	...	100	....	....	.....	20.0965	20,721	3.7003	.....	.....	.....	.....	76,788	1.017
4	30	66.679	171	84	79	178	3,240	20.5799	14,256	4.0772	18,054	3.6877	30,456	2.1236	66,096	1.008
5	28	66.225	168	86	85	173	4,536	14.8220	20,088	3.2967	11,664	5.6802	28,836	2.2906	65,124	1.016
6	19	52.277	154	....	....	....	.....	.....	.....	.....	10,378	5.0373	28,512	1.8440	53,136	0.983
7	15	39.690	146	70	66	155	3,240	12.2500	12,212	3.2500	8,748	4.5358	19,116	2.0746	43,416	0.916
8	15	49.215	154	72	72	159	3,565	13.8050	10,043	4.9004	8,748	5.0633	24,948	1.9727	47,304	1.040
9	14	40.824	141	71	66	....	3,565	11.4503	8,747	4.6070	8,100	5.0400	20,088	2.0322	40,500	1.008
10	14	34.473	141	66	62	143	2,916	11.8220	6,480	5.0111	7,128	4.8302	17,172	2.0075	33,696	1.020
11	14	33.339	139	65	58	143	3,175	10.5007	.....	.....	.....	.....	15,552	2.1437	31,752	1.049
12	13	30.164	135	65	62	139	3,240	9.0001	6,156	.....	6,480	4.6549	14,256	2.1158	30,132	1.001
13	12	29,937	141	67	58	138	2,592	11.5497	6,380	4.8099	5,932	5.0469	14,580	2.0532	29,484	1.010
14	11	31.708	137	68	59	141	2,916	10.9004	7,078	4.7049	6,480	4.7966	14,904	2.1335	30,780	1.038
15	9	31.071	....	64	61	....	.....	.....	4,860	4.9840	.....	.....	13,608	.....	.....	.....
16	8	26.308	174	60	59	....	.....	.....	.....	.....	.....	.....	11,340	.....	27,216	0.976

The last measurement, that of the head, is taken with the body immersed to the level of the prominence of the thyroid cartilage. The subject is told to hold his breath and duck slowly under the water, the assistant watching that head is completely under water. The oscillation of the column in the water-gage will go on for a while and settle in a few seconds. The observer at the water-gage notes the level and taps on the tank which is distinctly heard by the subject and is a signal that the experiment is at an end and that he may stand up. In my experiments I had every subject repeat the last procedure two or three times and the reading was found to differ in from 0.1 cm. to 0.2 cm. in some cases, and in others not at all. Of course it is interesting and highly instructive to take note also of the volume of the water displaced in deep inspiration and complete expiration, and compare it with the displacement during normal breathing. The whole procedure lasts from twenty to thirty minutes.

REMARKS ON THE AUTHOR'S EXPERIMENTS

The volumetric measurements were taken of fourteen individuals, four adults (three males and one female) and ten boys, ranging in ages between 53 and 8 years; in weight from 79 kg. to 26 kg., and in height from 1.71 meters to 1.35 meters. All the measurements were taken at 7 p. m. before the evening meal. The specific gravity of the entire body ranged between 0.976 and 1.049. The average specific gravity of the adults was 1.003, and of the boys 1.006. The average specific gravity of all individuals examined was 1.005. One of the subjects was placed on a certain diet and his displacement measured every other day, when he weighed 175, 174 and 173 pounds, respectively.[9]

As all my predecessors have made their experiments solely for the purpose of taking the specific gravity of the entire body, and therefore each person examined had nothing else to do on entering the filled tank but to immediately duck his head under the water, the observers encountered many difficulties which undoubtedly caused many errors in the calculations. The difficulty arises in having the subjects dip their heads during normal breathing. The tendency is, in the majority of cases, to take a deep inspiration, and, in a few cases, to make a complete expiration, before plunging. In the former case the reading will be too low, and in the latter case, too high. The subjects are frightened, anxious, excited. Not so with my method. The individual enters the bath and is first immersed to the navel line only, next to the nipple line, then to the prominence of the thyroid cartilage. He gradually accustoms himself to his new surroundings — to the tank and the water. Hearing each time the reading of the amount of water which

9. See table, author's observations.

each section of the body displaces, by the time the last dip is made, the immersion of the head, all fear and trepidation vanishes. In fact, all my subjects showed a lively interest in the experiment and were rather eager to take the last step in the experiment to find out what a "big head" they had. Especially interesting and amusing were the experiments with the boys, which were conducted in groups of two and three. They showed no less interest in the experiment than the observer himself. Only one little boy of 8 could not hold his breath long enough to permit the reading of the water level, but the little fellow made half a dozen attempts and was in tears when he did not succeed. Some of the readings are not recorded on our table because they were not correctly taken, or were not taken at all.

As all my predecessors[3] found the average specific gravity of the human body either too low (Robertson 0.8706, Suelzer 0.970, Herman 0.920) or too high (Krause 1.055, Ziegelroth 1.055, Miess 1.012, Meeh 1.012) as compared with the average specific gravity in our experiments (1.005), I consider it my duty to defend my results on the ground of their greater accuracy.

My tank has a smaller diameter than any of the vessels hitherto used, and because of the smaller diameter the errors in the volumetric measurements were necessarily smaller. Take, for instance, the tank of Robertson, whose dimensions were 183 cm. long and 76 cm. wide. An error in 1 cm. either way would make a difference in the result $(183) \times 76)$ of 13,908 c.c., a quantity equal in many subjects to the displacement of the volume of one-fourth of the whole body. In my tank (diameter 64 cm.) an error of 1 cm. would make a difference in the result $(32 \times 32 \times 3.14)$ of only 3,240 c.c., or one-fourth compared with the same error in Robertson's tank.

My observations have demonstrated beyond any doubt that in order to reduce the errors to a minimum, the tank must be made of the smallest possible diameter compatible with carrying out the experiment. The smaller the tank the smaller the error.

THE PONDERAL INDEX

One of the properties of living bodies is that of attaining a determinate development, whose limits, in regard to both the quantity of its mass and the harmony of its form, are defined by that biologic final cause which is implanted in the race and transmitted by heredity. These determinate limits constitute a fundamental biological property. One of its descriptive attributes are the limits to be ascertained by measure, namely, the mass of the body, and together with its mass, its anthropologic entirety.

The following measurements have been adopted by modern anthropometrists to determine the form: (1) the total stature; (2) the sitting stature; (3) the total spread of the arms; (4) the circumference of the thorax, and (5) the weight.

Of these measurements stature and weight are the most important. Without them we cannot get the true idea of the form of an individual. For instance, if two individuals have the same weight, 65 kg., for example, and one of them has a stature of 1.85 meters and the other 1.55 meters, it is evident that the first of these two will be very thin, because his weight is insufficient, while the second, on the contrary, will have an excessive weight. A stout, robust child will weigh less in an absolute sense, than an adult man who is extremely thin and emaciated; but relatively to the mass of his body, he will weigh more.

But here a difficulty arises. The linear measurements such as the stature cannot be compared with volumetric measurements such as the weight. It was Galton[8] who ingeniously cut the Gordian knot. He reduced the volumetric measure — the weight — to a linear measure by extracting the cube root from the number representing the weight. Then the root of the weight may be compared to the stature reduced to a scale of 100. This relative ratio between weight and stature is called the *ponderal index*. The formula may be represented as follows:

$H : \sqrt[3]{W} :: 100 : X$ (where H represents the height, W the weight, X the ponderal index);

Hence

$$\text{Ponderal index} = \frac{100\ \sqrt[3]{W}}{H}$$

This formula represents an index which is serviceable for practical purposes in the absence of a better one. But since the body does not consist of a homogeneous material I contend that the weight represents the sum of parts differing one from another, the difference in this instance being the specific gravity. For it makes a great difference whether a large proportion of the weight is adipose tissue, brain, or striped muscle. Each of the organs has its special specific gravity. It is evident, therefore, that neither the total weight of the body nor its stature, either separately or relatively, gives us an idea of its volume, less so of its constituent parts.

Let us take the case of two men weighing the same, say 64 kg., and measuring the same length, 150 cm. Both will have as a matter of course the same ponderal index ($Pi = \frac{\sqrt[3]{64}}{150} - 20.6$),[10] and yet their forms

10. Livi's Tables of Ponderal Index in Montessori's Anthropology.

may differ, because one is thin and makes up the weight in muscle, and the other is stout and makes up the weight in fat. The cubic root 4 of the weight 64 is after all an empirical number. On the contrary, by taking the weight of the human body in the air and then measuring the amount of water which the body displaces, we obtain two exact measurements, that of weight and that of volume, the ratio of which — the specific gravity — represents the natural and the only ponderal index of the human body.

I recognize the fact that with our present insufficient knowledge of the relative weight and specific gravity of each of the component parts of the human body, we cannot as yet construct a formula which, like that of Archimedes, would give us the respective quantities of the human alloy. And yet I believe that the time is dawning when the anatomists and the pathologists will pay more attention to the weight and specific gravity of every tissue and organ of the human body, and out of the thousands of observations certain definite laws will be deduced which will make it possible to tell from the weight, volume and stature the exact proportion of the weight of at least the three component parts, the musculature, fat and bone, just as it is possible to tell the weight of each component of an alloy if its total weight and the specific gravity of each component part is known.

It is evident that the factor of stature is of equal importance with weight and volume, and therefore the ponderal index which I advocate, namely, the specific gravity, must be based on the three measurements.

The following formula for a new ponderal index, which shall include the trinity of anthropometric measurements—weight, volume and stature, has been suggested by a friend:

Let P = ponderal index, W = weight, w = weight per unit volume, V = volume, H = height. Then, according to Galton's formula $P = \frac{100 \sqrt[3]{W}}{H}$; but since $W = Vw$, $P = \frac{100 \sqrt[3]{Vw}}{H}$. But $w = Sw_0$, where S = specific gravity and w_0 the weight per unit volume of standard substance (water), whence $P = \frac{100 \sqrt[3]{VSw_0}}{H}$. At 4° C., $w_0 = 0.9987$ grams per cubic centimeter, in other words w_0 is practically 1 gram, whence $P = \frac{\sqrt[3]{VS}}{H}$.

THE SPECIFIC EQUIVALENT GRAVITY

We have referred elsewhere to difficulty experienced by various observers in having their subjects dip their heads under water with the normal amount of air in their lungs.

Wengler[11] endeavored to obviate this difficulty by having the immersed person breathe normally while under water through a mask,

11. Virchow's Arch. f. path. anat., 1896, cxlvi, p. 456.

having an outlet from nose and mouth into the air. Meeh[12] thought he could obviate this difficulty by measuring the quantity of air inspired and expired by means of the spirometer, and then adding the result. Both procedures increase the technical difficulties. Jamin and Mueller[13] have therefore devised a method of procedure for the estimation of the specific gravity of the body which is free from the objections above referred to, which consists in taking the specific gravity of the body without obliging the person to dip his head under the water. Their procedure is as follows: The person to be measured is seated in the tank, the surface of the water being at the chin anteriorly and at the border of the hair-growth posteriorly. If we divide the weight of the body by the volume of water displaced when the body is in this position, Jamin and Mueller claim that the quotient, which practically differs from the specific gravity of the body, yet relatively furnishes a constant which gives the relation of weight to volume. The volume of the body, excluding the head, they named the *equivalent volume*. The quotient of the weight of the body and of the equivalent volume they call the *specific equivalent gravity*.

I have already stated that by following my method of gradual immersion beginning first with the umbilical line, then the nipple line, etc., I did not find any difficulty in having the subjects dip their heads while their lungs were filled with the normal quantity of air. I have adopted the specific equivalent gravity of Jamin and Mueller for the reason that the measurements of any part of the body will give constants and after the number of observations has multiplied will give, to use Davy's expression, "information curious and useful." I have indicated in the table the specific equivalent gravity in all experiments.

SUMMARY

1. Next in importance to the weighing of the body in the air comes the measuring of the body in water. The first gives us the idea of the mass and the second of the volume. One is not complete without the other.

2. The smaller the diameter of the apparatus the more accurate will be the results. The future apparatus must have the smallest diameter compatible with carrying out the measurement of the volume of the body.

12. Meeh: Ztschr. f. Biol., 1879, xv, 425.
13. München. med. Wchnschr., 1903, i, 1454.

3. It is possible to measure the volume of separate sections of the body.

4. Our conception of the ponderal index must be changed. It should consist primarily of the ratio of weight to volume and refer secondarily to stature

5. The further study of the subject of specific gravity will reveal many new and valuable facts which will have a practical bearing on biology, anthropometry and metabolism.

6. I venture to prophesy that the armamentarium of the future physician will include next to the scales, a specific gravity apparatus.

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BIBLIOGRAPHY

Specific Gravity of the Entire Body.

Baumgartner, A.: Die Mechanik in ihre Anwendung auf Künste und Gewerbe, Wien., 1834, p. 94, quoted by Valentin, *q. v.*

Dalton: Frorieps Notizen für Natur- und Heilkunde, Erfurt, 1832, xxxiv, 236; quoted by Valentin, *q. v.*

Dapper: Ueber den Stoffwechsel bei Entfettungskuren, Beitraege zur Lehre vom Stoffwechsel des gesunden und kranken Menschen von Prof. v. Noorden, Berlin, 1904, part 2, A. Hirschwald.

Herman: Quoted by Buhl in Mitt. a. d. pathol. Inst. z. München., 1878, p. 4.

Huschke: Soemmering Lehre von die Eingeweiden und den Sinnesorganen des menschlichen Körpers. Umbearbeit und geendigt von G. Huschke, Leipzig, 1844, pp. 10, 11. Quoted by Valentin, *q. v.*

Jamin, F., and Muller, E.: Ueber das spezifische Gewicht des lebenden Menschen mit besonderer Berücksichtigung eines für klinische Zwecke brauchbaren spez. Aequivalentgewichts, München. med. Wchnschr., 1903, I, 1454-1457.

Krause: Handbuch der menschlichen Anatomie., 1879, Ed. 3, p. 12.

Lahman: Physiatrische Blättern., Leipzig, 1893, Ed. 2; quoted by Ziegelroth, *q. v.*

Mech, K.: Oberflächemessungen des menschlichen Körpers, Ztschr. f. Biol., 1879, xv, 425.

Mech: Volumenmessungen des menschlichen Körpers und seine einzelnen Theile in den verschiedenen Alterstuffen, Ztschr. f. Biol., 1894, new series, xiii, 125.

Mishnewsky, V.: Kopredieleniu udelnaho viesa tiela chelovieka, San. Dielo, St. Petersburg, 1892, iii, 3.

Mies: Virchow's Arch. f. path. Anat., 157, 1899, clvii, 98.

Mies: Ueber die Höhe und die Höhenzahl des Gewichts und des Volumens von Menschen und Tieren, Virchows Arch. f. path. Anat., 1891, cxxiii, 188.

Muller, E., and Seidelmann, W.: Ueber das Verhalten des spezifischen Körpergewichtes bei gesunden Mannschaften, Med. Klin., Berl., 1906, xi, 213-215.

Langer, C.: Ueber Form und Massverhältniss des menschlichen Körper, Wien. med. Wchnschr., 1880, xxx, 1357.

Packard, John C.: Specific Gravity of the Human Body, Scientific Am. Supplement, 1910, xlix, 20382.

Platt, Julia B.: On the Specific Gravity of Spirostomia, and the Tadpole, Am. Naturalist, 1909, xxxiii, 38.

De Parville, H.: La densité de l'homme, Nature, 1896-7, xxv, Part 1. p. 283.

Rubner, M.: Beiträge zur Ernährung im Knabenalter mit besonderer Berücksichtigung der Fettsucht, Berlin, 1902, A. Hirschwald.

Quetelet: Anthropométrie, 1870.

Robertson, John: Philosophical Transaction, l, Art. 5; quoted in Hutton's Philosophical and Mathematical Dictionary, London, 1815 (Article, Specific Gravity).

Suelzer: Verhandl. d. Cong. f. inn. Med., Third Congress, 1844, p. 370.

Valentin, G.: Lehrbuch der Physiologie des Menschen, Braunschweig, 1851, i.

Vierordt, H.: Anatomische Physiologische und physicalischer Tabellen, Jena, G. Fischer, 1896, Ed. 3.

Wengler, J.: Ein Versuch, das specifische Körpergewicht am lebenden Menschen zu bestimmen, Wien. med. Wchnschr., 1902, lii, 1739; 1799.

Wengler, J.: Bestimmung des spezifischen Körpergewichtes am lebenden Februar, 1902, Deutsch. med. Wchnschr., 1902; Soc. Proc., p. 119.

Wengler, J.: Bestimmung des spezifischen Korpergewichtes am lebenden Menschen, München. med. Wchnschr., 1903, i, 2094.

Wengler, J.: Das Volumen and spezifische Gewicht des menschlichen Körpers, insoweit er aus festen Stoffen bestehet, also unter Ausschluss der variablen Körperluft, Pfluger's Arch. f. d. ges. Physiol., 1906, xcv, 612.

Williams, Stephen: The Specific Gravity of Some Fresh Water Animals in Relation to their Habits, Development and Composition, Am. Naturalist, 1909, xxxiv, 9.

Ziegelroth: Das spezifische Gewicht des menschlichen Körpers und Blutes, zugleich ein Beitrag zur Krasenlehre, Virchows Arch. f. path. Anat., 1896, cxlvi, Part 3, p. 453.

Zuntz, N.: Eind Methode zur Schätzung des Eiweiss- und Fettgehaltes im lebenden Tierkörper, Vertrag in der physiologischen Gesellschaft zu Berlin vom 17 Nov., 1912, Deutsch. med. Wchnschr., 1902, Soc. Proc. p. 338.

Specific Gravity of Separate Parts of the Human Body

Baistrocchi: Riv. sper. di fren. e di med. leg., 1884, p. 193.

Bischoff: Sitzungsb. der K. bayer, Akad. d. Wissensch. zu München, 1864, xi, 347.

Chenevix: Tr. Am. Pharm. Soc., 1803, p. 195.

Danielewsky: Centralbl. f. d. med. Wissensch., xviii, 1880, p. 241.

Davy: Tr. Med.-chir. Soc., Edinburgh, 1829, iii, 436.

Davy: On the Specific Gravity of the Human Body, Researches Physiological and Anatomical, London, 1839, ii, 253.

Fricke: Versuche über den spezifischen Gewicht des menschlichen Körpers, Freiburg, 1832.

Giacosa: Arch. p. le sc. med., 1882, vi, 29.

Harless: Wagner's Handwörterbuch der Physiologie, 1853, iv, 512.

Huschke: Soemmering's Lehre von den Eingeweiden und Sinnersorganen umgearbeit von Huschke, 1844, v, 310.

Kapff: Untersuchungen über das specifische Gewicht thierischer Substanzen, Tübingen Dissertation, 1832.

Krause: Handbuch der menschlichen Anatomie, 1879, Ed. 3, p. 950.

Krause and Fischer: Ztschr. f. ration, Med., 1866, Series 3, xxvi, 306.

Lyons: Report on the Pathology of the Diseases of the Army in the East, 1856.

Michel and Wagner: Arch. f. Ophth., xxxii, Bd., 1886, Suppl. 2, xxxii, 155.

Nunnelly: Quart. Jour. Microscop. Sc., 1858, p. 138.

Obersteiner: Anleitung zum Studium des Baues der nervösen Centralorgane im gesunden und kranken Zustande, 1892, Ed. 2, p. 134.

Schatz: Arch. der Heilk., 1876, xvii, 529.

Smidt, H.: Ueber das spezifische Gewicht der Leber und der Milz, Virchow's Arch. f. path. Anat., 1880, lxxxii, 1.

Toldt: Studien über die Anatomie der menschlichen Brustgegend, 1875, p. 66.

BOOK REVIEW

CHEMICAL PATHOLOGY. Being a Discussion of General Pathology from the Standpoint of the Chemical Processes Involved. By H. Gideon Wells, Ph.D., M.D., Professor of Pathology in the University of Chicago and in Rush Medical College, Chicago. Second Edition, thoroughly revised. Cloth, pp. 616. Price $3.25 net. Philadelphia and London: W. B. Saunders Company, 1914.

A comparison of the second edition of Dr. Wells' chemical pathology with the first edition, published seven years before, gives interesting evidence of the lines along which the chemical study of disease has progressed and an equally striking demonstration of the scantiness of our knowledge of the exact chemical nature of the complex substances concerned in the production of disease and in the organism's defense against it. Dr. Wells does not confine himself to a statement of actual chemical knowledge of pathological lesions and processes in the strict sense, but gives a short survey of the chemistry and physics of the cell, of the nature and action of enzymes and anti-enzymes, and of bacteria and animal parasites and the poisons produced by them, as well as the poisons manufactured by the higher animal organisms.

In these earlier chapters one notes as new especially the discussion of the doubly refractive lipoids and the myelins of the cell, the recent acquisitions in knowledge of anti-enzymes, including Abderhalden's theories, and studies of catalase and anticatalase. There is much new material on autolysis and a very interesting discussion of the correlation of histological and chemical changes in autolysis.

The chapters dealing with the chemistry of the immunity reactions, while excellent short presentations of present knowledge and theories, show most clearly how little these essentially chemical phenomena have been reduced to chemical terms. For the most part these chapters might have appeared equally well in a book on immunity or general pathology, not written from the chemical point of view. The substances involved are still almost wholly hypothetical and of unknown chemical constitution. Vaughan's protein split products approach nearest to definite chemical entities. Friedberger's so-called anaphylatoxin and Abderhalden's *Abwehrfermente* receive consideration. These chapters are placed in a new position in the book and are brought wholly up to date. The discussion of the body's chemical defense against non-protein poisons shows a comparatively small advance in knowledge.

Then follow chapters dealing with the various pathological processes. That on inflammation is to a small extent chemical. In the discussion of circulatory disturbances and diseases of the blood the chief addition to chemical knowledge has been the study of the H-ion concentration by Rolly's gas chain method and Henderson's simpler ones. The most recent theories of fibrin formation, including Howell's, are discussed, but here again, as in the immunity reactions, real chemical substances have not been isolated.

In the chapter on edema the recent studies of colloid chemistry, including Fischer's theory, receive consideration. Of the actual composition of edematous fluids, however, nothing more seems to be known. The nature of the retrogressive pathological changes has received much more satisfactory chemical elucidation, and the discussion of the relation of the lipoids to fatty metamorphosis shows that real progress has been made. For the clinician a fuller presentation of the work on cholesterol in the blood and tissues would have been desirable. The exact chemical nature of amyloid appears as much of a riddle as ever.

The chapter on calcification, concretions and incrustations has been largely rewritten, and as this is a field in which Dr. Wells has himself made large contributions, the discussion is especially excellent.

Little new has been added to the knowledge of pathological pigments, except in the case of ochronosis and the iron pigments.

In the study of tumors chemists have been especially active, and new knowledge on tumor enzymes, hemolytic substances produced by neoplasms and the immunity reactions of tumors is well given. In their chemical composition the study of the fatty constituents of hypernephromas is the chief advance.

The last four chapters deal with metabolic abnormalities or diseases. Uremia and eclampsia receive considerable attention, and the knowledge derived from the study of the blood during life by clinicians is the main evidence of progress. The actual chemical causes of these intoxications, however, are still undiscovered. Acid intoxication and diabetic coma are well presented. Dr. Wells still holds to beta-oxybutyric acid as the mother substance of the acid bodies, though recognizing from Dakin and Wakeman's work the probability that the conversion of beta-oxybutyric and aceto-acetic acids into one another is reversible. Evidence seems to be accumulating that in all probability aceto-acetic acid is the actual precursor of beta-oxybutyric acid. The chapter as a whole has been rewritten and brought up to date. The newer studies of the inborn errors of metabolism, alkaptonuria and cystinuria, which have been so thoroughly described by Garrod, are well discussed. The so-called gastro-intestinal autointoxication receive very sane treatment.

In the domain of the ductless glands the chief contributions to chemical knowledge concern the thyroid, but the clinical work is satisfactorily presented in brief, Cushing's studies of the hypophysis being especially notable. Wells maintains a properly critical attitude toward the different problems presented by the adrenals.

A few additions to the real knowledge of uric acid formation and destruction and of the uric acid content of the circulating blood by Folin's new colorimetric method have made the problem of the causation of gout no more intelligible. The concluding chapter on diabetes by Woodyatt is an illuminating presentation of facts and theories and adds greatly to the value of the book.

One misses any reference to the pathological problems of nutrition. The pathology of growth, of obesity or of the nutritional or deficiency diseases which are at the present time receiving so much study is not mentioned. Vitamins would seem as much entitled to a place in chemical pathology as precipitins or opsonins, and one would hope that in a future edition Dr. Wells might expand the scope of his work to include these fields, in which the chemical study of metabolism is proving especially fruitful. He also fails to include the pathological alterations in the total metabolism as revealed by direct and indirect calorimeter studies of fever. The book is especially strong therefore in the presentation of chemical post-mortem pathology — that is, chemical pathological anatomy—though presenting satisfactorily those portions of the chemical pathology of disease in the living which are considered. It is a most valuable reference book for the clinician or pathologist interested in the chemical study of disease and contains an excellent bibliography.